EFFECT OF DIFFERENT FORMS OF IODINE ON LAYING HENS

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FOUR FIGURES

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The seasonal variation in the iodine content of the thyroid of birds and mammals has been well established. Recently much interest has been aroused by the discovery that the iodine content of eggs can be influenced by the diet of the hen. According to Wilder, Bethke and Record ('33) the amount of iodine in the egg depends on the amount of iodine in the diet but is not influenced by the form in which it is fed to the bird.

Obviously, the feeding of iodine to laying hens raises a number of questions regarding the effect of different forms and amounts of iodine on the birds, their eggs and their progeny. In order to answer some of these questions, a series of experiments has been conducted. This paper presents the results of some of these experiments designed to determine the effects of different amounts of iodine and of iodine from different sources on laying hens.

In a preliminary experiment the birds were divided into four lots of which three lots, each comprising four laying hens, were given by mouth the following: 1, desiccated kelp (two capsules containing 815 γ of iodine a day); 2, desiccated thyroid (one capsule containing 815 γ of iodine a day) and 3, potassium iodide solution (2 cc. containing 815 γ of iodine

[1] We are indebted to Dr. J. W. Givens for many of the iodine analyses reported in this paper.

a day). In the case of lot 4, potassium iodide solution was injected subcutaneously into two hens. The birds fed desiccated thyroid lost weight and stopped laying. The two hens into which potassium iodide solution was injected also lost weight, but continued to lay. The kelp and the potassium iodide solution (given by mouth) apparently had no effect on body weight or egg production.

To test further the effect of iodine from different sources on laying hens a ration was made up of ground yellow corn 48 pounds, ground wheat 24 pounds, yeast 2 pounds, alfalfa meal 5 pounds, dried skim milk 10 pounds, meat scrap 5 pounds, ground limestone (calcite) 5 pounds, salt 0.5 pounds, and sardine oil 0.5 pounds. This ration contained 50 γ of iodine in 100 gm. of the ration.

Thirty-five hens in their second laying year were divided into five lots. The number of hens in each lot together with the amount of iodine fed in addition to the iodine in the basal ration is shown in table 1. Lot 1 received the basal ration only (iodine content 50 γ per 100 gm. of feed). For lot 2 oyster shell was substituted for the calcite in the basal ration which increased the iodine content to 250 γ per 100 gm. of feed. Lot 3 received the basal ration plus 800 γ of iodine in the form of desiccated thyroid fed by capsule to each bird daily. The desiccated thyroid fed the first 3 weeks contained 0.308 % iodine while that fed from the fourth to eleventh weeks, inclusive contained 0.174 % iodine. Lot 4 received the basal ration plus 2 cc. daily of an aqueous iodo-salicylic acid solution containing 800 γ of iodine. At the end of the sixth week, the amount of iodo-salicylic acid solution was doubled. Lot 5 received the basal ration plus 2 cc. daily of an aqueous sodium iodide solution containing 8000 γ of iodine for the first 6 weeks and 4 cc. (16,000 γ of iodine) daily for the remaining 5 weeks. The iodo-salicylic acid and sodium iodide solutions were introduced into the crop by pipette. All analyses were made by the method described by Almquist and Givens ('33).

TABLE 1

Iodine in the yolk and albumen of eggs in γ per egg

EXPERIMENT NO.	SOURCE OF IODINE OTHER THAN THE BASAL RATION	NUMBER OF HENS	AMOUNT OF ADDITIONAL IODINE GIVEN THE HENS DAILY, γ	EXPERIMENTAL PERIOD (NUMBER OF WEEKS)									
				1	2	3	4	5	6	7	8	9	11
2	None	11		≦3	≦3	≦3	≦3	≦3	≦3	≦3	≦3	≦3	≦3
2	Oyster shell[1]	12		≦3	38	35	42	42	42	42	35	39	42
2	Desiccated thyroid[2]	4	800	≦3	145	....	....	400	126	116	74	830	85
2	Iodo-salicylic acid	4	800[3]	≦3	≦3	≦3	10	61	125	180	420	50	40
2	Sodium iodide	4	8000[3]	300	1190	1520	1510	1730	1300	1050	1512	...	1350
				1 to 4 weeks	5 to 8 weeks	9 to 10 weeks	11 to 12 weeks	13 to 16 weeks	17 to 20 weeks				
3	Iodo-salicylic acid	4	800	19.0	21.1	25.4	102.0	4.7	10.0				
3	Sodium iodide	4	800	54.8	44.3	44.5	70.0	11.7	94.9				
				6th week	8th week	11th week	14th week	17th week	25th week				
4	None	4	See text	10	10	10	15	20	10				
4	Potassium iodide	4		125	125	85	120	120	120				
4	Potassium iodate	4		130	105	120	130	100	100				
4	Iodo-salicylic acid	4		30	10	30	...	20	10				
4	Di-iodotyrosine	4		30	105	30	125	100	100				
4	Iodized olive oil	4		25	75	50	30	30	30				
4	Decissated thyroid (0.3 % I_2)	4		30	65	35	50	30	...				

[1] Five pounds of oyster shell was substituted for the calcite in the basal ration.

[2] See text.

[3] Amount doubled at the end of 6 weeks.

In the third experiment, two groups of four hens each were given the same basal ration. Lot 1 received 1 cc. (800 γ of iodine) of iodo-salicylic acid, lot 2, 1 cc. (800 γ of iodine) of sodium iodide. Since the amount of iodine fed was small, only the iodine analysis of eggs, excreta and glands will be considered. In a fourth experiment seven groups of four hens were used. The same ration was fed to each group. One group received this ration only while the other six groups were given the iodine preparations shown in table 1 in such amount as to add 14,000 γ to each kilogram of feed.

EXPERIMENTAL RESULTS

The fluctuations in the average weight of the birds in experiment 2 are shown in figure 1, the per cent egg production in figure 2 and the feed consumed by three of the five groups in figure 3. In general, feed consumption varied with changes in egg production and body weight. There was relatively little variation in the case of the birds fed the unsupplemented basal and oyster shell rations, although body weight decreased temporarily at the end of the third week. The birds given iodo-salicylic acid likewise varied relatively little after an initial decrease in weight and a temporary decrease after the amount of iodo-salicylic acid was increased at the end of the sixth week.

The birds fed desiccated thyroid ate very little the first 3 weeks. They lost weight rapidly and stopped laying. After changing the desiccated thyroid preparation fed, about the end of the third week, the birds consumed more feed, gained in weight and started to lay despite the fact that their iodine intake was the same as before. It should be noted that in this case the preparation with the higher percentage of iodine was more active per unit of iodine.

After a period of a little over 1 week, the birds fed sodium iodide decreased in weight. Egg production was apparently not affected, but when the amount of sodium iodide was doubled at the end of the sixth week, both body weight and egg production decreased. Feed consumption decreased the

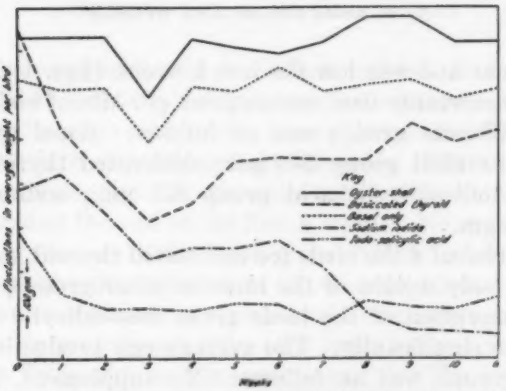

Fig. 1 Fluctuations in average weight (in grams) per bird.

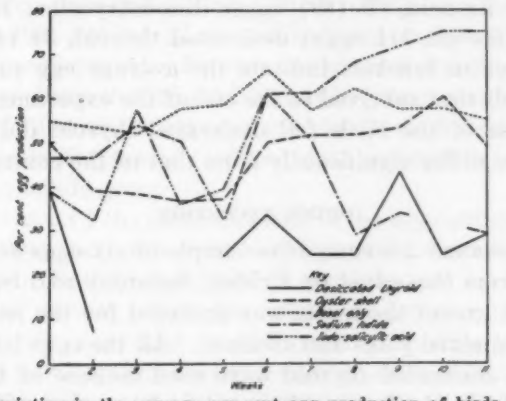

Fig. 2 Variation in the average per cent egg production of birds given iodine from different sources.

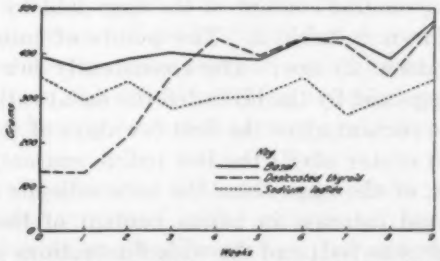

Fig. 3 Amount of feed consumed (in grams) each week per kilo of body weight.

first 4 weeks and was low the last 4 weeks (figs. 1, 2 and 3). The average weekly feed consumption per kilo of body weight for the different groups was as follows: Basal group 402 gm.; oyster shell group 349 gm.; desiccated thyroid group 324 gm.; iodo-salicylic acid group 375 gm.; sodium iodide group 308 gm.

In experiment 4 the birds fed desiccated thyroid lost weight while the body weight of the birds in other groups, with the possible exception of the birds given iodo-salicylic acid, did not change significantly. The average egg production of the various groups was as follows: No supplement, 91 eggs; potassium iodide, 113 eggs; potassium iodate, 103 (128) eggs; iodo-salicylic acid, 69 (89) eggs; di-iodotryosine, 133 eggs; iodized olive oil, 111 eggs; desiccated thyroid, 34 (44) eggs. The figures in brackets indicate the average egg production of the birds that survived to the end of the experiment. Only in the case of the birds fed desiccated thyroid did the egg production differ significantly from that of the controls.

IODINE EXCRETION

In experiment 2 a composite sample of six eggs selected at random from those laid on Friday, Saturday and Sunday of each week except the tenth, was analyzed for the iodine content of the mixed yolks and albumen. All the eggs laid by the birds fed desiccated thyroid were used because of the small number obtained. In experiments 3 and 4 a composite sample was taken for analysis at 2- to 8-week intervals.

The average iodine content of the eggs laid by the various groups is shown in table 1. The points of interest in this table (experiment 2) are: The consistently low iodine content of the eggs laid by the birds fed the basal ration only; the higher iodine content after the first few days of the eggs from the birds fed oyster shell; the low iodine content, during the first 4 weeks, of the eggs from the iodo-salicylic acid group; the more rapid increase in iodine content of the eggs when sodium iodide was fed; and the wide fluctuations in the iodine content of the eggs from the groups receiving desiccated

thyroid, iodo-salicylic acid or sodium iodide. It should also be noted that there was a temporary increase in the iodine content of the eggs laid by the hens given iodo-salicylic acid during the sixth, seventh and eighth weeks. Most of this increase occurred after the amount of iodo-salicylic acid given was doubled at the end of the first 6 weeks. No such increase occurred in the case of the birds given sodium iodide although the amount of iodine given was likewise doubled.

The results obtained in experiment 3 with an equal intake of iodine from iodo-salicylic acid and sodium iodide are similar to those obtained in experiment 2. The iodine content of the eggs from the iodo-salicylic acid group increased more slowly than the iodine content of the eggs from the sodium iodide group and remained lower except during the 11- to 12-week period. In experiment 2, the iodine content of the eggs from the iodo-salicylic acid group was relatively less, except during the seventh and eighth week period, when it was relatively greater (iodine intake considered) than for the sodium iodide group.

In experiment 4 the iodine content of the eggs obtained from any one group was quite consistent but there was a significant difference in the iodine content of the eggs laid by the birds in different groups. Thus the difference between the iodine content of the eggs laid by the birds given the potassium iodide and iodo-salicylic acid was over nine times the standard error of the difference. It is evident that the iodine content of the eggs laid by the birds fed potassium iodide, potassium iodate and di-iodotyrosine was about the same. The eggs from the birds given iodized olive oil and desiccated thyroid were intermediate in iodine content while the iodine content of the eggs from the birds receiving iodo-salicylic acid did not differ significantly from that of the eggs laid by the control group.

In order to determine what variation there was in the amount of iodine contained in the excreta, two analyses were made of composite samples from the different groups in experiment 2 and at 4-week intervals in experiment 3. As shown

in table 2, the percentage of iodine in the excreta of the birds fed the basal ration only and those fed oyster shell was low and did not differ appreciably. A higher percentage of iodine was found in the excreta from the birds given iodo-salicylic acid than from the group fed desiccated thyroid (experiment 2) although both received the same amount of iodine. In experiment 2 the excreta from the birds receiving sodium iodide contained the highest percentage of iodine but the increase over the other groups was not in proportion to the greater amount of iodine given these birds. In experiment 3 the excreta of the birds fed iodo-salicylic acid contained a

TABLE 2

Iodine content of the excreta in γ per gram

IODINE SUPPLEMENT GIVEN	EXPERIMENT 2		EXPERIMENT 3					
	3rd week	6th week	Before	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
None	3.75	10.1						
Oyster shell	2.01	6.98						
Desiccated thyroid	79.5	22.3						
Iodo-salicylic acid	86.5	131	5.09	4.22	42.6	4.13	5.64	2.3
Sodium iodide	394	253	0.24	3.55	16	0.30	1.73	1.9

higher percentage of iodine than those of the birds given sodium iodide. The results obtained in experiments 2 and 3 thus indicate that the excreta of the birds fed iodo-salicylic acid contained a higher percentage of the total iodine intake than those of birds fed sodium iodide.

Differences in the excretion of iodine were studied further in two balance experiments which were run in order to determine whether there was a difference in the percentage of the total iodine intake recovered in the feces and in the eggs laid. The feed consumed during a period of 1 week was weighed, the feces collected and a composite sample of the feces, and of the eggs laid, analyzed for iodine. The data are summarized in table 3. So far as the iodine content of the eggs is concerned, the results agree with those presented in

table 1. The analyses of the feces also agree with those in table 2 in that the highest percentage of iodine was found in the feces of the birds given iodo-salicylic acid while less was recovered from the feces of the birds fed potassium iodide and least from the feces of the birds fed di-iodotyrosine or 48 % in both trials. If the percentage of iodine in the excreta of the birds given iodo-salicylic acid is compared with that of the birds given potassium iodide or sodium iodide it will be observed that the differences are so consistent that there can be little doubt that more of the iodine fed in the form of iodo-salicylic acid is excreted in the feces.

TABLE 3

Summary of iodine balance trails (experiment 4)

TRIAL	SOURCE OF IODINE	FEED CONSUMED	TOTAL IODINE IN FEED	NUMBER OF EGGS LAID	IODINE PER EGG	TOTAL IODINE IN EGGS	TOTAL IODINE IN FECES	TOTAL IODINE EXCRETED	RECOVERY
		gm.	γ		γ		γ	γ	%
1	Potassium iodide	2120	29,700	18	120	2160	17,200	19,360	66
1	Iodo-salicylic acid	1300	18,200	10	20	200	12900	13,100	71
1	Di-iodotyrosine	2640	37,200	18	125	2250	18000	20,250	55
2	Di-iodotyrosine	2990	42,000	19	125	2400	20,000	22,400	54

IODINE IN THE TISSUES

At the end of experiments 2 and 3 representative birds from the groups on the basal and oyster shell ration and all the birds left of the other three groups were killed. Analyses made of the thyroids, ovaries and parts of the thymus of these birds are shown in table 4. It will be observed that the percentage iodine content of the thyroids differs from what might be expected on the basis of the amount of iodine fed, the thyroids of the hens receiving sodium iodide containing only slightly more iodine, on the average, than those of the hens fed the basal ration. There were considerable differences between individuals hence, small differences between the groups cannot be considered significant. The thymus contained an appreciable amount of iodine while the ovaries

(stripped, so far as possible, of all ova) contained very little. The ovaries of the hens given sodium iodide contained significantly more iodine than the others (experiment 2). This was apparently due to the high iodine intake since the iodine con-

TABLE 4

Iodine content of the thyroid, thymus and ovary in γ per gram of material

EXPERIMENT NO.	IODINE SUPPLEMENT GIVEN	THYROID			THYMUS		OVARY	
		Number of birds	Iodine content		Number of birds	Average iodine content	Number of birds	Average iodine content
			Average	Range				
2	None	3	1540	1430– 1,710	1	54	1	7
2	Oyster shell	5	1950	830– 4,350	2	1190	1	1
2	Desiccated thyroid	2	3570	1490– 5,650	2	104	2	5
2	Iodo-salicylic acid	4	4920	2610–10,700			2	3
2	Sodium iodide	4	1880	930– 3,590			2	32
3	Iodo-salicylic acid	3	390		1	60	3	1
3	Sodium iodide	4	530		3	49	4	4

Fig. 4 Thyroid of a bird fed the basal ration only (4 a), and of a bird fed the basal ration plus sodium iodide (4 b) (experiment 2). × 8.

tent of the ovaries from the sodium iodide group in experiment 3 was low.

Histological examination of the thyroids confirms in a general way the analytical results. The lobules in the thyroids of birds fed the basal ration were smaller than the lobules in the thyroids of the birds fed sodium iodide as will be seen by comparing figures 4 a and b.

Table 5 shows the results of iodine analyses of the spleen, pancreas, kidney, fat and blood. The iodine content was low and variable. Thus the relatively high iodine content of the pancreas of the birds given iodo-salicylic acid was due to the

TABLE 5

Iodine in γ per gram of material (experiment 3)

	IODO-SALICYLIC ACID GROUP		SODIUM IODIDE GROUP	
	Number of birds	Average	Number of birds	Average
Spleen	3	1.3	4	2.93
Pancreas	3	3.3	4	0.90
Kidney	3	0.3	4	0.20
Fat	3	1.23	4	0.35
Blood	3	0	4	1.38

high value obtained for one bird only. While the iodine content of the thymus, and other tissues is extremely variable, the data presented show that iodine may occur in appreciable amounts outside the thyroid.

DISCUSSION

The results summarized above show that the feeding of moderate amounts of desiccated thyroid decreased feed consumption, body weight and egg production. The loss in body weight observed agrees with the findings reported by others (Hutt, '30; Asmundson, '31). The effects of thyroid feeding observed do not apparently depend upon the total amount of iodine given in the form of desiccated thyroid, which agrees with the conclusion of Morch ('29) that the activity of the preparation is not closely related to the iodine content. Since

the effects of desiccated thyroid are due to the thyroid hormone it is not to be expected that other iodine preparations would have a similar effect. Nevertheless, when sodium iodide is fed in large amounts the apparent effect is the same as in the case of desiccated thyroid—feed consumption is decreased, after which follows loss of weight and decrease or cessation of egg production.

The amount of iodine in the egg varies according to the form in which it is given to the birds. Thus the iodine content of the eggs laid by birds (experiment 4, table 1) fed sodium iodide, sodium iodate and di-iodotyrosine was significantly higher than that of the eggs from the birds fed iodo-salicylic acid. These results are in agreement with those obtained in experiments 2 and 3 although the results of the earlier experiments were not so clear cut. Considering all the data there can be no doubt that, contrary to the results reported by Wilder, Bethke and Record ('33), the form in which the iodine is given does influence the amount of iodine in the eggs laid. It follows from this that if the iodine in the various rations is from different sources, the iodine content of the eggs will not necessarily vary according to the amount of iodine fed. When, however, the same form of iodine is fed at different levels it might be expected that the amount of iodine in the eggs would vary with the amount of iodine in the feed. Contrary to this the data in table 1 for birds fed sodium iodide at different levels (experiments 2 and 3) indicate that the iodine in the egg does not vary in direct proportion to the iodine intake. Our results, while not conclusive on this point, agree with those reported by Almquist and Givens ('35) and disagree with those of Wilder, Bethke and Record ('33).

The data in tables 1, 2 and 3 indicate that if the iodine content of the excreta is high, or the percentage of the total iodine intake recovered in the feces is high, the iodine content of the eggs is relatively low. The percentage of iodine recovered from the feces was higher when iodo-salicylic acid was fed than when potassium iodide or di-iodotyrosine was

fed. The results of the various analyses also point to the conclusion that relatively more of the iodine is excreted when it is fed in the form of iodo-salicylic acid than if sodium or potassium iodide are fed.

The percentage of iodine in the thyroid may also vary with the source since there was relatively less storage of iodine in the thyroids of the birds fed sodium iodide than in the case of the birds given iodo-salicylic acid or desiccated thyroid. The percentage of iodine in the thyroids of the birds fed oyster shell, desiccated thyroid and sodium iodide was about the same as reported by Cruickshank ('29) for laying hens while the values for the basal group were lower and those for the birds receiving iodo-salicylic acid were higher. When the iodine intake from iodo-salicylic acid and sodium iodide was low (experiment 3) the results were somewhat different. Evidently, many factors influence the iodine content of the thyroid, hence no definite conclusions can be drawn with respect to the effect of feeding different forms of iodine on the amount of iodine in the tissues, until more extensive data are available.

SUMMARY

White Leghorn hens were supplied with iodine from oyster shell, desiccated thyroid, sodium iodide, potassium iodide, iodo-salicylic acid, di-iodotyrosine and iodized olive oil. The birds were weighed at regular intervals and iodine determinations were made on samples of eggs from all the groups and on the feces and the thyroids and other tissues from some of the groups.

The body weight, egg production and feed consumption was not adversely affected except in the case of the birds fed desiccated thyroid and in the case of the birds given sodium iodide when they received 16,000 γ of iodine per bird daily.

The iodine content of the eggs varied from 3 to 1730 γ per egg. It differed according to the source of the iodine but corresponded only approximately to the iodine intake. Thus the iodine content of the eggs laid by birds fed potassium

iodide, potassium iodate and di-iodotyrosine was significantly higher than the iodine content of the eggs laid by the birds fed iodo-salicylic acid. When iodo-salicylic acid was fed a higher percentage of the total iodine ingested was excreted then when di-iodotyrosine and potassium (or sodium) iodide was fed. These results indicate that iodine utilization by laying hens, in so far as it can be measured by the iodine content of the eggs and the excreta, depends upon the source as well as the amount of iodine given.

The iodine content of the thyroid varied considerably but the differences were not statistically significant. The thymus also contained appreciable amounts with less iodine in the ovary (stripped of yolks), spleen and other tissues.

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VITAMIN C STUDIES WITH CHILDREN OF PRESCHOOL AGE

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ONE FIGURE

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The recently developed chemical methods for determining with considerable accuracy the amount of vitamin C (ascorbic acid) in biological material have made possible studies of vitamin C retentions in children. Hitherto, in the few studies pertaining to the ascorbic acid needs of children, comparisons have been made between the amount ingested, as orange juice, and the urinary output. In some cases, this has been estimated from urine samplings; in others, from the 24-hour excretions. Without knowledge of the ascorbic acid content of all the foods eaten, it is obvious that actual needs may be higher than the figure thus obtained. Furthermore, the method of preserving the urines in some of the studies suggests that deterioration of the ascorbic acid may have occurred, thus accounting for certain of the low values.

The purpose of the present study was to determine how much vitamin C is needed by children of the preschool age. The investigation was made with three normal boys aged 39, 57 and 59 months, respectively, at the beginning of the study. During the investigation the children lived under controlled conditions, as described in a previous report (Daniels et al., '34). Each study period consisted of 15 days, a 5-day preliminary period for physiological adjustment, and two 5-day balance periods. During the preliminary period also, retentions were determined but since the urinary ascorbic acid

output was apt to be irregular, depending, it would seem, upon the degree of saturation of the tissue of a given child, these balances have not been included.

The food served was prepared in three lots, one for the preliminary and one for each of the two balance periods. Cooked foods were stored in closely covered porcelain containers in a low temperature refrigerator (8°C.), a weighed portion being removed each day for serving. The orange juice, which contained considerable pulp, was prepared at the beginning of each period by extracting both juice and pulp with a glass hand press and subsequently straining through a wire (16 mesh) sieve. This was immediately stored in air-tight jars in the low temperature refrigerator.

The ascorbic acid content of the foods was determined by means of the 2:6 dichlorophenolindophenol method described by Birch, Harris and Ray ('33), with modifications suggested by Tauber and Kleiner ('35) and Ahmad ('35), 10 gm. samples of the more solid foods and 30 gm. samples of the daily aliquot milk mixtures being used in each case. The foods were thoroughly ground with purified sea sand, and 10 to 15 cc. of 10 % trichloracetic acid, the extracted material being decanted into a large centrifuge tube. Second and third extractions were similarly made. In all, 45 cc. of the trichloracetic acid were used for each sample of material. The combined extractions were centrifuged at high speed, the clear solution was decanted, made to a definite volume and titrated immediately against a standard solution of 2:6 dichlorophenolindophenol, the average of six titrations being taken as the final value. The ascorbic acid content of orange juice diluted 1:10 was similarly determined. Tests of the cooked foods at the beginning and end of the metabolism period, and of the orange juice each day indicated that under the conditions of storage no deterioration of vitamin C had occurred.

Throughout the period of study, which was continuous from September 29th to February 15th, with the exception of 4 days between each controlled period, and the Christmas holidays, the kind of food, method of preparation, and with few exceptions, the amounts served to each child were the same during

each of the several periods. Foods were eaten quantitatively. Twice only was it found necessary to increase the caloric ingestions, each time increases being made with those foods (apple sauce, potatoes and stick candy) which contained little or no vitamin C. The daily orange juice was divided into two portions and given between meals. To this were added 3.7 cc. of a standard cod liver oil and 4 drops of viosterol. A typical

TABLE 1

Food and vitamin C intake of children, January 14th to 20th

	G.E.		F.V.		J.E.	
	Amount	Ascorbic acid	Amount	Ascorbic acid	Amount	Ascorbic acid
	gm.	*mg.*	*gm.*	*mg.*	*gm.*	*mg.*
Orange juice	60.0	43.6	60.0	43.6	60.0	43.6
Banana	61.0	11.9	76.0	14.9	61.0	11.9
Milk	732.0	5.9	732.0	5.9	732.0[1]	5.9
Green beans	70.0[1]	2.4	70.0[1]	2.4	90.0[1]	3.1
Potato	80.0[1]	2.5	80.0[1]	2.5	80.0[1]	2.5
Carrots	56.0[1]	1.8	56.0[1]	1.8	75.0[1]	2.4
Prunes	20.0[1]	0	53.3[1]	0	53.3[1]	0
Cereal, dry weight	13.8	0	13.8	0	13.8	0
Bread	49.0	0	51.0	0	51.0	0
Butter	15.0	0	18.0	0	17.0	0
Egg	80.0	0	110.0	0	110.0	0
Sugar	21.0	0	21.0	0	19.0	0
Beef	40.0	0	60.0	0	70.0	0
Apple sauce	70.0[1]	0	91.0[1]	0	70.0[1]	0
Cod liver oil	7.5	0	7.5	0	7.5	0

[1] Weight and ascorbic acid content after cooking.

day's food intake for one period, January 14th to 20th, with the ascorbic acid value of each food served, is given in table 1. Variations in the amounts of vitamin C were obtained by increasing the orange juice, and by adding to the orange juice just previous to serving a solution of commercial ascorbic acid[1] as indicated.

Urines were collected quantitatively, each voiding being preserved with 2 cc. of toluene and from 2 to 4 drops of concentrated sulphuric acid, depending upon the amount of

[1] Redoxon, obtained from Hoffmann-La Roche, Inc., Nutley, N. J.

orange juice taken, thus keeping the pH between 2.7 and 3. Urine specimens were placed immediately on ice, in a closed chest, and subsequently (twice each day) were transferred to a low temperature refrigerator (5°C.). At the end of each 24 hours, the urines were pooled and the ascorbic acid content, by means of the 2:6 dichlorophenolindophenol method was determined with unfiltered urines, since it had been shown that filtering under the conditions tested resulted in some destruction of the ascorbic acid contained therein. Tests of urines to which known amounts of commercial ascorbic acid were added gave recoveries of 97 to 102 %.

That the methods used for preserving and storing the urines were satisfactory and allowed for no appreciable destruction of the ascorbic acid during the collection periods is indicated by comparisons of tests made after the urines had been stored 24, 48 and 72 hours, respectively. Tests with the same urine similarly stored, but preserved with glacial acetic acid, confirmed the findings of Johnson and Zilva ('34) to the effect that some destruction occurs within 24 hours; this increases with longer storage. Urines preserved with sulfuric acid in quantities to bring the pH to 2.7 to 3 were found to be stable at least 90 hours, the longest testing period.

Comparative tests of the ascorbic acid content of urines preserved as indicated with sulfuric acid by the two methods recommended: 1) titration against 2:6 dichlorophenolindophenol (Birch, Harris and Ray, '33); and 2) colorimetric determinations with phospho-18-tungstic acid (Medes, '35), which rules out possible phenols and thiol compounds were found to be in close agreement, the range of difference (—6.5 to + 9.6 %) being within the limits of error of the methods.

RESULTS

The ascorbic acid retentions of the children studied have been grouped in order of ingestions per kilogram of body weight, including only those values obtained subsequent to the adjustment periods. Since, in some instances, a fair proportion of the ascorbic acid intake was obtained from foods other

than orange juice and commercial ascorbic acid when used, the amount of the vitamin from the several sources is listed separately. When only 1 ounce of orange juice (22 ± mg. of ascorbic acid) was given, slightly more than one-half of the total vitamin C intake was obtained from the other foods served; when 4 ounces of orange juice (90 ± mg. of ascorbic acid) were taken, from one-fourth to one-fifth was furnished from other sources.

The ascorbic acid ingestions ranged from 2.7 to 12.7 mg. per kilogram of body weight. Following the 5-day preliminary period, the urinary excretions, which were significantly constant from day to day, tended to parallel the ingestions. In no case were the very low excretions reported by other workers (Hess and Benjamin, '34; Harris and Ray, '35) found, 10.6 mg. being the lowest obtained in any metabolism period. This followed an ingestion of 3.1 mg. per kilogram with the youngest child studied (G.E., 12/15).

Retentions based on the assumption that all excess of intake is excreted through the kidneys, ranged between 1.7 and 4.3 mg. per kilogram, and in general, paralleled the ingestion up to 7.5 mg., or thereabout per kilogram. With the highest ingestions tested, 10.7 to 12.7 mg. per kilogram, no more vitamin C was retained than with the next lower level tested, namely, 7.5 mg. per kilogram, suggesting that this latter amount is the minimum for children of the ages studied. Depleted tissues will retain more until they become saturated. This is shown by the daily urinary excretion of the three children immediately following the 3-week holiday period at home (table 3). With a total ingestion of 68.1 mg., G.E. excreted an average of 11.1 mg. per day during 5 successive days of the preliminary period, whereas during the following 6-day period, he excreted an average of 20.4 mg. F.V. and J.E. also excreted somewhat less during these preliminary days, suggesting that in all three cases the home diets of the children contained less than the optimum amount of vitamin C. On the other hand, when a diet fairly high in vitamin C followed one which had been shown to be adequate (table 4) the day-by-day urinary excretion remained constant during the period of study.

TABLE 2

The relation of ascorbic acid ingestion to ascorbic acid retention

NAME	DATE	WEIGHT	CREATININE	INTAKE						URINARY EXCRETION		RETENTION		
				Orange juice	Food	Redoxon	Total	Per kilogram	Per milligram creatinine	Average	Range	Total	Per kilogram	Per milligram creatinine
		kg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
J.E.	12/15	19.0	339	22.5	29.4	..	51.9	2.7	0.15	14.6	13.0– 15.8	37.3	2.0	0.11
F.V.	12/15	19.3	293	22.5	30.7	..	53.2	2.8	0.18	15.6	13.3– 17.6	37.6	2.0	0.13
G.E.	12/15	16.3	245	22.5	28.0	..	50.5	3.1	0.21	11.6	10.6– 13.3	38.9	2.4	0.16
J.E.	1/19	19.2	346	38.9	25.0	..	63.9	3.3	0.19	25.0	19.0– 37.5	38.9	2.0	0.11
F.V.	1/19	18.6	294	38.9	25.1	..	64.0	3.4	0.22	17.2	15.4– 20.3	46.8	2.5	0.16
J.E.	1/14	19.2	365	43.6	25.8	..	69.4	3.6	0.19	36.1	24.2– 52.8[1]	33.3	1.7[1]	0.09[1]
J.E.	9/29	18.0	318	48.0	18.8	..	66.8	3.7	0.21	20.3	13.8– 26.6	46.5	2.6	0.15
J.E.	11/26	18.9	348	48.0	23.7	..	71.7	3.8	0.21	29.7	26.7– 35.3	42.0	2.2	0.12
G.E.	1/19	16.2	254	38.9	22.6	..	61.5	3.8	0.24	17.8	17.1– 18.9	43.7	2.7	0.17
F.V.	11/26	18.7	297	48.0	25.1	..	73.1	3.9	0.25	26.5	24.0– 28.2	46.6	2.5	0.16
F.V.	1/14	18.4	287	43.6	27.4	..	71.0	3.9	0.25	16.7	13.3– 19.7	54.3	3.0	0.19
J.E.	12/ 1	19.0	343	48.0	27.2	..	75.2	4.0	0.22	29.5	27.8– 30.0	45.7	2.4	0.13
F.V.	12/ 1	18.7	296	48.0	28.2	..	76.2	4.1	0.26	25.8	25.0– 27.3	50.4	2.7	0.17
F.V.	9/29	16.8	272	48.0	20.5	..	68.5	4.1	0.25	23.0	21.0– 24.0	45.5	2.7	0.17
G.E.	1/14	16.2	249	43.6	24.5	..	68.1	4.2	0.27	20.2	15.2– 25.5	47.9	2.9	0.19
G.E.	9/29	14.9	232	48.0	18.8	..	66.8	4.5	0.29	23.2	13.6– 29.3	43.6	2.9	0.19
G.E.	11/26	15.8	249	48.0	22.9	..	70.9	4.5	0.28	21.5	19.7– 24.0	49.4	3.1	0.20
G.E.	12/ 1	16.0	251	48.0	25.7	..	73.7	4.6	0.29	22.8	21.5– 25.8	50.9	3.2	0.20
J.E.	10/20	18.3	323	45.0	18.1	48	111.1	6.1	0.34	45.9	41.4– 50.0	65.2	3.5	0.20
J.E.	10/15	18.3	347	47.2	19.1	48	114.3	6.2	0.33	57.8	50.0– 66.7	56.5	3.1	0.16
J.E.	11/10	18.6	350	90.0	30.7	..	120.7	6.4	0.34	56.2	52.2– 61.5	64.5	3.5	0.18
J.E.	11/ 5	18.6	350	96.0	22.7	..	118.7	6.4	0.34	51.3	51.1– 53.3	67.4	3.6	0.19
F.V.	10/20	17.5	286	45.0	20.2	48	113.2	6.5	0.40	58.4	51.1– 66.7	54.8	3.1	0.19
F.V.	11/ 5	18.1	295	96.0	24.2	..	120.2	6.6	0.41	51.6	51.1– 52.2	68.6	3.8	0.23
F.V.	10/15	17.3	286	47.2	21.2	48	116.4	6.7	0.41	66.6	61.3– 75.0	49.8	2.9	0.17
F.V.	11/10	18.2	293	90.0	32.8	..	122.8	6.7	0.42	56.0	54.5– 57.1	66.8	3.7	0.23
G.E.	10/20	15.2	239	45.0	17.2	48	110.2	7.3	0.46	56.0	52.2– 60.0	54.2	3.6	0.23
G.E.	10/15	15.1	240	47.2	18.7	48	113.9	7.5	0.47	56.4	50.0– 61.5	57.5	3.8	0.24
G.E.	11/ 5	15.5	242	96.0	20.7	..	116.7	7.5	0.48	51.2	48.0– 54.5	65.5	4.2	0.27
G.E.	11/10	15.6	243	90.0	27.7	..	117.7	7.5	0.48	54.3	51.1– 57.1	63.4	4.1	0.26
J.E.	2/ 9	19.4	335	87.3	24.3	96	207.6	10.7	0.62	142.4	140.8–145.4	65.2	3.4	0.19
F.V.	2/ 9	19.1	293	87.3	25.0	96	208.3	10.9	0.71	149.5	144.0–156.0	58.8	3.1	0.20
J.E.	2/ 4	19.3	333	90.0	26.5	96	212.5	11.0	0.64	148.1	137.1–160.0	64.4	3.3	0.19
F.V.	2/ 4	19.0	288	90.0	27.8	96	213.8	11.3	0.74	151.2	143.4–157.9	62.6	3.3	0.22
G.E.	2/ 9	16.7	255	87.3	23.2	96	206.5	12.4	0.81	134.2	128.0–143.2	72.3	4.3	0.28
G.E.	2/ 4	16.6	255	90.0	25.4	96	211.4	12.7	0.83	147.6	141.2–160.0	63.8	3.8	0.25

[1] Child had slight cold with elevated temperature 1 day. Increased excretion of creatinine during 1 day, and vitamin C during 2 days. Two and five-tenths grams of aspirin given two successive nights.

The highest retentions, 4.1, 4.2 and 4.3 mg. per kilogram, following ingestions of 7.5, 7.5 and 12.4 mg. per kilogram, respectively, were obtained with the youngest child, G.E. (39 months of age at the beginning of the investigation). That these high retentions were not found with the other children,

TABLE 3

Daily intake and excretion of vitamin C following a 3-week period of home diet

DATE	G.E.	F.V.	J.B
	Intake: 68.1 mg.	Intake: 71.0 mg.	Intake: 69.4 mg.
	Urinary vitamin C	Urinary vitamin C	Urinary vitamin C
	mg.	*mg.*	*mg.*
January 8	11.3	12.2	18.9
9	11.1	10.6	19.5
10	9.4	14.5	18.1
11	10.4	14.6	23.8
12	13.2	19.0	27.5
13	23.4	15.2	27.0
14	23.5	13.3	25.5
15	21.0	17.1	24.2
16	20.0	17.1	....
17	15.2	16.4	....
18	19.5	19.7	....

TABLE 4

Urinary excretion of vitamin C during a high intake and a subsequent lower intake

F.V.			
Intake: 122.79		Intake: 73.09	
Date	Urinary vitamin C	Date	Urinary vitamin C
	mg.		*mg.*
11/10	57.14	11/20	28.23
11/11	55.38	11/21	28.08
11/12	54.54	11/22	28.62
11/13	55.81	11/23	23.29
11/14	57.14	11/24	27.45

although seemingly a plethora of vitamin C was given during certain periods, suggests that young tissues may have greater need for vitamin C than more mature tissues. Hess and Benjamin found that infants excreted very little vitamin C even though they were receiving diets which would seem to contain adequate amounts. "Only when excessively large doses

of vitamin C were fed (1 pt. orange juice daily) did appreciable urinary excretion occur." In seeming corroboration of the possible greater demand of young tissues are the findings of Bessey and King ('33) to the effect that in younger animals the vitamin C content of the tissues was higher than that of older animals. The human tissues tested also were found to contain more vitamin C than those of the adult (Bessey and King, '33; Yavorsky, Almaden and King, '34).

Estimating vitamin C retentions on the basis of creatinine eliminations of the children studied, further suggests that the younger child retains more than the older (table 2). G.E., with a creatinine elimination of 255 mg., retained 0.25 and 0.28 mg. of ascorbic acid per milligram of creatinine during two successive periods (2/4 and 2/9, respectively) when receiving the highest ingestions, whereas F.V., with creatinine eliminations of 288 and 293 mg. retained 0.22 mg. and 0.20 mg. per milligram of creatinine, respectively, while J.E. (2/4 and 2/9) with the highest creatinine elimination, retained only 0.19 mg. of vitamin C per milligram of creatinine, when receiving what would seem to be considerably more than sufficient to cover requirements. This greater demand of the younger tissue for vitamin C may be concerned with the more rapid oxidation processes of this age. The fact that during anesthesia there is an increased output (Zilva, '35; Bowman and Muntwyler, '35) also points to a relationship between oxidation needs and vitamin C. More work is needed to confirm these findings.

That commercial ascorbic acid can be used for supplying the vitamin C need of human tissues has been indicated by a number of investigators (Svensgaard, '34; Goettsch, '35; Hawley, Stephens and Anderson, '36). Further confirmation of this is shown in the balance studies with the children, when synthetic ascorbic acid was used as part of the vitamin requirement. The total ingestions of this period (10/15) were comparable to those of the subsequent period (11/5) in which the vitamin was supplied wholly from food. Urinary excretions and retentions of ascorbic acid were very similar during

both periods; whereas retentions were considerably lower, between 1.7 and 3.1 mg. per kilogram, respectively, during the periods (9/29, 11/26 and 1/14) in which similar amounts of vitamin C were furnished from foods, but none from the synthetic ascorbic acid. The higher retentions of the former periods would seem to be due to the increase in the ascorbic acid, part of which was furnished by the commercial preparation.

COMMENTS

The similarity of response of the three children receiving comparable amounts of ascorbic acid at various levels of ingestion would seem to attest to the validity of the findings

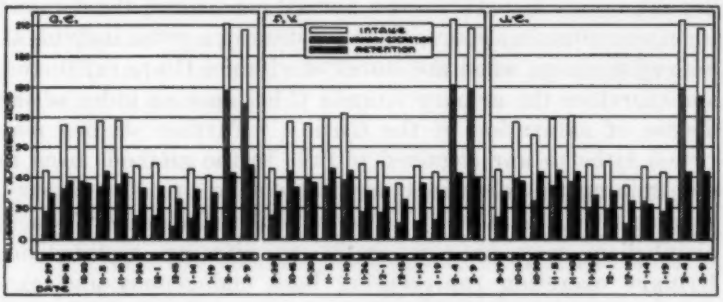

Fig. 1 The relation of intake to urinary excretion and retention of vitamin C in children.

(fig. 1). With the exception of the very highest levels tested, retentions were directly related to ingestion, indicating some rather definite relationship between vitamin C and tissue needs. At all levels of ingestion, urinary excretions tended to parallel ingestions. The explanation for this is not clear. When less than optimum amounts are taken for any considerable period, it would seem that tissue demands would reduce excretion to a minimum, whereas even at the lowest levels of ingestion tested, ascorbic acid was found in appreciable amounts. Tests for interfering thiol compounds (Shinohara and Padis, '36) indicated that at most only approximately 3 mg. of ascorbic acid equivalent could be thus

accounted for—too little to explain the difference in the urinary excretions. During each period of study, the children received the same foods in the same amounts with the exception of orange juice and the commercial ascorbic acid. Thus the urinary excretion of any interfering substances should be the same during all periods for a given child. Nor does it seem probable that the method of giving the ascorbic acid so flooded the system that less would be retained during some periods than others. The orange juice was always given under the same conditions, therefore storage with all levels of ingestion would be equally possible.

Urinary excretion is believed to represent excess over needs. What then is the explanation for excretions with the low retentions tested? Under normal conditions, the demand for tissue vitamin apparently is constant in a given individual. Scurvy develops when the stores of vitamin C are exhausted, and therefore the urinary vitamin C becomes an index of the degree of saturation of the tissue. "Further studies may reveal hitherto unrecognized pitfalls in the methods used in determining the vitamin C content of the urine" (Hawley, Stephens and Anderson). On the otherhand, ascorbic acid metabolism may be comparable to nitrogen metabolism: Urinary excretion representing both endogenous and exogenous acid. Until the tissues become completely exhausted, the endogenous vitamin C will always appear in the urine, high urinary outputs with less than optimum ingestions being due to the fact that the organism adjusts slowly to different levels of ingestion.

SUMMARY

In an attempt to determine the vitamin C needs of young children, three boys of preschool age were given during twelve successive periods constant weighed diets differing from period to period only in the amount of ascorbic acid contained therein. A 5-day preliminary period for physiological adjustment preceded each two consecutive 5-day metabolism periods. During four periods, a part of the vitamin was supplied by a synthetic commercial preparation.

1. Under the conditions of the investigation, urinary excretion of ascorbic acid paralleled the intake, notwithstanding the fact that retentions at certain levels of ingestion were considerably below the physiological optimum.

2. Retentions of ascorbic acid paralleled the ingestions only up to 7.5 mg. or thereabout per kilogram. Higher ingestions (10 to 12 mg. per kilogram) were without influence on the retentions of the children studied.

3. The highest retentions estimated either on the basis of weight (3.8 to 4.3 mg. per kilogram), or creatinine elimination (0.24 to 0.28 mg. per milligram of creatinine), were obtained with the youngest child, suggesting that there is a greater demand by younger tissue for more vitamin C.

4. Comparable amounts of commercial ascorbic acid and ascorbic acid from foods resulted in similar retentions.

5. The relation of ascorbic acid intake to excretion and retention is discussed.

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STUDIES ON THE RELATION OF DIET TO GOITER

IV. THE ANTIGOITROGENIC VALUE OF SOME FOODS

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ONE FIGURE

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According to evidence accumulated over more than 80 years, enlarged thyroid glands are most frequent in those populations whose environment is relatively deficient in iodine. Goitrous areas have been reported to exist in practically every country. Attempts have been made to arrive at some value for the daily human iodine requirement by analysis of food and water from goitrous and non-goitrous regions, or from the daily iodine excretion of persons living in such regions. Results are conflicting, but a probable value is 50 to 100 γ per day. Lunde ('29) found daily elimination of 40 γ at Hosstvedt, Norway (60 % goiter in children), one of 61 γ at Ruud (30 % goitrous) and 173 γ at Viki Sojn, where goiter is absent. He believes that the daily human requirement is at least 50 γ, possibly as high as 100 γ. Oppenheimer ('29) concluded from the work of Fellenberg and others that the average human requirement from all sources is 100 γ per day. Mazzocco ('29) calculated that the average daily intake of inhabitants of Salta, Argentina, a goitrous area, is less than 40 γ. In one of the previous papers of this series (Levine, Remington and von Kolnitz, '33 b), it was shown that goiter could be effectively prevented in young rats by supplying 1 to 2 γ

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of iodine per day as iodide, and that this corresponded, on a basis of caloric intake, to 60 to 120 γ for the human. There is quite an extensive literature on the iodine content of food, water, and soil from all parts of the world.

Until quite recently it has been assumed that, with respect to trace elements (e.g., iron, copper, manganese, iodine, etc.), the nutritional value of foods was indicated by chemical analysis. Some have even gone farther than this, and argued that these elements as found in food of plant or animal origin, should be more readily and completely utilized in the diet, than inorganic forms. That neither of these generalizations is wholly valid is shown, with respect to iron, by Elvehjem ('32), who reported that the iron of hematin is less completely utilized than that of ferric chloride, and by Sherman, Elvehjem and Hart ('34 b), who found the iron of spinach, oysters, alfalfa, and blood only 25 % available. The amount available for hemoglobin building in the rat paralleled very closely the amount present in inorganic form as determined by the dipyridyl method. These same authors ('34 a) found the copper of egg yolk inferior to copper sulfate. The calcium of spinach is very poorly utilized as compared with that of milk, according to a recent report by Fincke and Sherman ('35). With regard to iodine, the physiological requirement for which is much lower than that of any other known inorganic factor, few and inconclusive experiments have been made. Fellenberg ('23), using himself as a subject, and a very low iodine intake, reported that retention in the body is in the following descending order: Iodized fats, iodides, iodine in vegetables, iodine in animal foods. Pfeiffer ('29) raised radishes on an iodine-enriched soil, and fed the leaves, parallel to potassium iodide, to dogs. He found greater retention in the body from iodide, but of the iodine retained, much more was stored in the thyroid when radish leaves were fed.

In the process of developing our technique for the study of low iodine goiter in the rat (Remington and Levine, '36) to a point where it is possible to evaluate the response to different forms or compounds of iodine, we have fed different

iodine-bearing foods for the purpose of estimating their efficiency in goiter prevention. In this paper are reported preliminary results on some sea foods, and on dried milk. Coulson ('35) has recently redetermined the iodine content of a number of edible sea fish and crustaceans. Among these, haddock was found to be extremely high in iodine, showing an average value, fresh basis, of 5130 parts per billion in the muscle. Coulson ('34) has also made iodine analyses on sixty-four samples of Atlantic and Gulf Coast oysters, the average of which was 492 parts per billion, fresh basis. Samples of these products, which had been dried, ground and analyzed, were on hand in the laboratory. We also had available samples of dried milk from cows in a non-goitrous region,

TABLE 1

Iodine content of supplements fed (parts per billion)

High iodine milk no. 1020	900
High iodine milk no. 1221	1170
Oysters	3200
Haddock	32600
Irish moss	350000
Low iodine milk	ca. 190–260

and prepared by roller process in a semi-commercial plant (Remington and Supplee, '34), and in which analysis had shown a relatively high iodine content.[3] Samples of these products were therefore used in the experiments herein reported, their iodine content being shown in table 1.

EXPERIMENTAL

Four series of feeding experiments were run on different dates, using our goitrogenic diet GP (Levine, Remington and von Kolnitz, '33 a) as a basal diet, details and results being shown in table 2. In series I and II, the products to be tested were incorporated in the diet at the levels shown, the completed diets analyzed and the average daily iodine intake calculated from the food consumption. In series III the

[3] Special Dryco manufactured by the Dry Milk Company at Newberry, S. C.

TABLE 2

The goiter-preventing value of certain iodine-containing foods. Experimental period 35 days

	SUPPLEMENT FED	IODINE INTAKE	NUMBER OF RATS	INITIAL AND FINAL WEIGHT	FRESH THYROID WEIGHT	DRY MATTER IN THYROID	IODINE IN THYROID DRY BASIS
		γ per day		*gm.*	*mg. per 100 gm. body weight*	%	%
Series I	None	0.13	9	61–114	23.9 ± 0.88	22.7	0.015
	2.8 % low iodine milk	0.18	9	59–133	19.8 ± 0.59	24.4	0.021
	0.86 % oysters	0.35	9	62–122	16.7 ± 0.53	25.2	0.038
	20.43 % low iodine milk	0.44	9	59–169	15.5 ± 0.58	25.9	0.053
	2.80 % high iodine milk	0.37	9	60–141	15.5 ± 0.52	26.1	0.059
	6.45 % oysters	2.09	9	61–152	13.2 ± 0.26	27.0	0.114
	20.43 % high iodine milk	2.27	9	59–176	11.0 ± 0.37	28.7	0.113
	Potassium iodide	3.06	9	60–115	12.1 ± 0.34	31.3	0.200
Series II	None	0.13	8	63–133	24.2 ± 1.30	22.6	0.010
	Potassium iodide	3.20	6	60–111	14.3 ± 0.52	29.9	0.285
	5.50 % oysters	1.98	8	56–137	14.1 ± 0.54	25.6	0.135
	15.00 % high iodine milk	2.14	8	64–172	13.6 ± 0.40	27.3	0.138
	15.00 % low iodine milk	0.53	8	58–161	18.5 ± 0.70	23.7	0.065
Series III [1]	None	0.15	15	58–123	29.2 ± 1.34	21.5	0.022
	0.16 gm. oysters	0.65	8	60–153	16.8 ± 0.81	25.5	0.041
	0.32 gm. oysters	1.15	8	64–170	14.9 ± 0.84	26.1	0.062
	0.64 gm. oysters	2.15	8	60–163	14.8 ± 0.43	27.1	....
Series IV [1]	None	0.15	9	56–129	24.4 ± 0.67	20.0	0.034
	0.38 % haddock	1.45	9	55–127	10.1 ± 0.28	32.8	0.113
	0.76 % haddock	2.70	9	55–126	9.6 ± 0.59	31.5	0.142
	0.036 % Irish moss	1.46	9	55–123	16.4 ± 1.20	26.2	0.066
	0.072 % Irish moss	2.65	9	53–116	14.1 ± 0.45	28.7	0.086
	Potassium iodide	2.80	8	57–119	10.7 ± 0.42	32.6	....

[1] In series III and IV the basal diet was not analyzed, but 0.15 γ per day was added to the iodine yielded by the supplement.

oysters were fed separately, daily, in weighed doses. In series IV the dried haddock and Irish moss were added to the basal diet in the proportions shown in the table, and the iodine calculated on the assumption that the basal diet contained 15 parts per billion. Since the amounts of dried milk necessary to yield a preventive dose of iodine were rather high, we also fed samples of commercial milk powder from a more goitrous region, and which was relatively low in iodine.[4] This low iodine milk was not analyzed, but its iodine content was calculated from that of the mixed diets containing it.

In the third paper of this series (Remington and Levine, '36) it has been shown that in low iodine goiter in the rat, certain relationships exist between the iodine content of the thyroid and its fresh weight, and also between dry matter content and weight, have drawn curves showing these relationships, and have proposed that in any given experiment, we are dealing with an iodine deficient condition if the points fall on these two curves. For the purpose of demonstrating how this may be done, and substantiating our argument as to the goiter-preventing potency of these foods, we have reproduced in figure 1 the curves from the previous paper, on which we have placed the points for the values obtained in the present series of experiments. As can be seen, the agreement is excellent, and establishes not only that the enlarged glands obtained are due to iodine deficiency, but also that the reduction in size obtained by feeding iodine-bearing foods is parallel to the storage of iodine, and decrease of edema, in the glands.

For the purpose of evaluating the relative antigoitrogenic properties of foods as compared with one another, or with iodides, it is necessary to show some relationship between amount of iodine fed, and either iodine content of gland or size of gland. In the earlier stages of our work it was hoped that we could establish permanent reference curves for these relations, which would be valid in all subsequent experiments where the same basal diet was used. As previously pointed

[4] Klim, manufactured by The Borden Company, at Perry, N. Y.

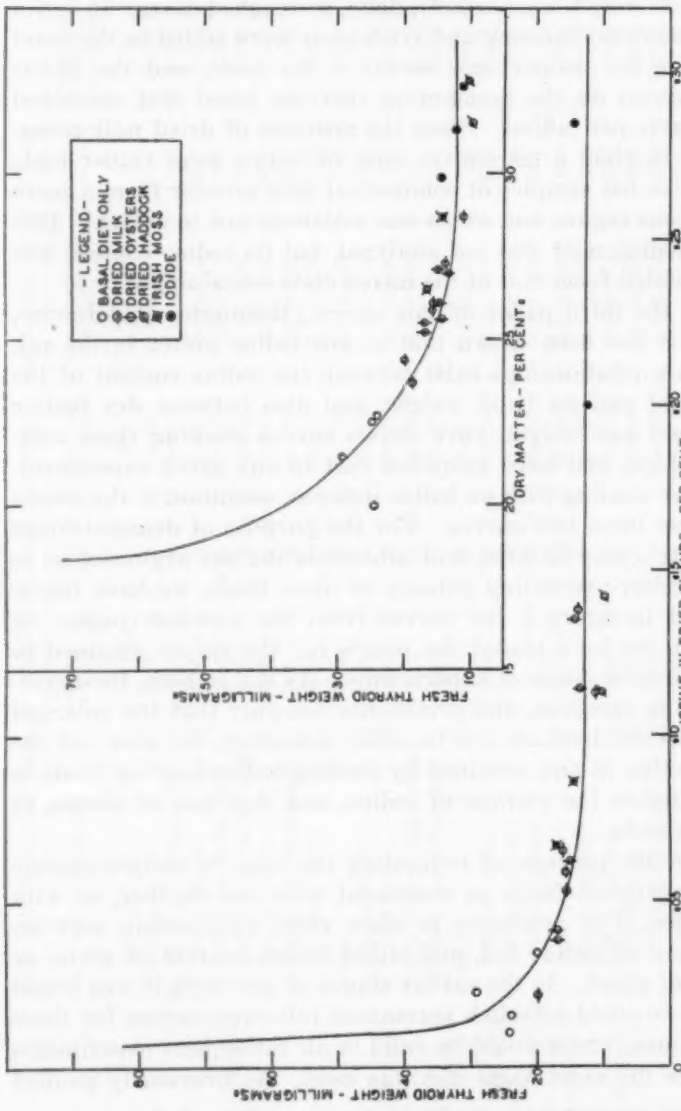

Fig. 1 Showing correlations between thyroid size and iodine content, and thyroid size and dry matter content, in rats fed iodine-bearing foods. Thyroid weight expressed in milligrams per 100 gm. body weight.

out, however, the iodine requirement of the rat is so low, and unknown disturbing factors so difficult to guard against, that the only rational basis of comparison seems to be to place a group of animals on the unsupplemented basal diet in parallel with each experiment, using the same batch of diet, and keeping all other factors as nearly constant as possible.

The experiments reported here were begun before we had arrived at this conclusion, and hence simultaneous iodide feeding within the critical range was not done. The evaluation of these foods in terms of iodide can therefore be deduced only in a general way by comparison with our previously reported experiments on iodine requirement. In that work, rats on the unsupplemented diet produced glands averaging 50 mg. per 100 gm. body weight, whereas in the present work they weighed only 24 to 29 mg., the difference, we believe, being due to the animals securing approximately 0.1 γ per day of iodine from an unknown source. Rats of the earlier series, receiving 0.48 γ of iodine per day from basal diet and iodide, yielded glands weighing 18.4 mg. per 100 gm. body weight, of 25.1 % dry matter and 0.049 % iodine. Comparing these values with those obtained for milk and oysters in series I and II where the *known* iodine intake was 0.35 to 0.44 γ per day, we find that the glands are somewhat smaller (15.5 to 16.7 mg.), the iodine content (0.038 to 0.059 %) and the dry matter content (25.2 to 26.1 %) of about the same order of magnitude. The results indicate that the thyroid response to iodine as it occurs in oysters and milk is of the same order of magnitude (within about 20 %) as that to iodide, and that the claimed superiority in the utilization of 'food iodine,' when fed at minimal effective doses, is not supported by these experiments.

We can also compare the different foods used in a given experiment with one another, and can draw conclusions as to whether or not a given food possesses goiter-preventing properties which may be correlated with its iodine content. For instance, in series I and II, which were done at approximately the same time, and in which the negative controls showed the same degree of goiter, we can compare oysters,

high iodine milk, and low iodine milk, at a daily intake level of approximately 0.4 γ of iodine, and again at about 2.0 γ. Such a comparison is shown in table 3, an inspection of which leads to the following conclusions:

1. The goiter preventing properties of oysters and milk are in proportion to their iodine content.

2. No significant differences in the availability of the iodine in oysters and milk are observed.

3. The response to a given dosage of iodine in the form of oysters or milk is of the same order of magnitude as that which we found in our earlier work ('33 b) for potassium iodide.

TABLE 3

Effect of practically equivalent dosages of iodine in the form of milk and oysters

IODINE INTAKE	TEST SOURCE OF IODINE AND AMOUNT USED IN DIET	THYROID WEIGHT	DRY MATTER IN THYROID	IODINE IN DRY THYROID
γ per day		*mg. per 100 gm. body weight*	%	%
0.35	Oysters 0.86 %	16.7 ± 0.53	25.2	0.038
0.37	High iodine milk 2.80 %	15.5 ± 0.52	26.1	0.059
0.44	Low iodine milk 20.43%	15.5 ± 0.58	25.9	0.053
0.53	Low iodine milk 15.0 %	18.5 ± 0.70	23.7	0.065
2.09	Oysters 6.45 %	13.2 ± 0.26	27.0	0.114
1.98	Oysters 5.50 %	14.1 ± 0.54	25.6	0.135
2.27	High iodine milk 20.43 %	11.0 ± 0.37	28.7	0.113
2.14	High iodine milk 15.0 %	13.6 ± 0.40	27.3	0.138

The protein of our basal diet, derived from wheat gluten and corn meal, is not of the highest biological value, and we have considered the possibility of this protein inadequacy having a part in the production of enlarged glands. This question, we believe, is answered by the results of series I and II. In series I it was necessary to feed 20% of low iodine milk in order to give about as much iodine as was contained in 2.8 % of high iodine milk. Nevertheless, the thyroid response was the same in both cases, *and corresponded to the iodine intake.* In series II, where high iodine and low iodine milk are fed at the same level, there is a marked and significant difference in thyroid response.

THYROID SIZE AND BODY WEIGHT

Animals on the higher levels of milk and oysters made much better growth than did those on basal diet. This may have been due to improved protein, or increased mineral and vitamin supply, or both. On our GP diet growth is retarded, averaging about 10 to 12 gm. per week. When 20 % of dried milk or as little as 3 % dried oysters were added, this growth rate nearly doubled. In our goiter work heretofore we have calculated thyroid weight to 100 gm. body weight, in order to have a uniform basis of comparing different groups of animals. Growth on our diet supplemented by iodide or by very small quantities of iodine-rich foods is fairly constant, so that for such animals this method of comparison is justified. In the present work, however, rats on higher levels of milk and oysters made greater growth, reaching weights of 160 to 170 gm., instead of 120 to 130 gm., during the experimental period. This raises the question as to whether, in these cases, expressing thyroid weight in terms of 100 gm. body weight gives an accurate comparison.

According to Donaldson ('24) and McCarrison ('30), the relation of thyroid weight to body weight of normal rats between 100 and 200 gm. is nearly, but not quite linear but is not a constant. McCarrison's values, expressed in terms of 100 gm., are 9.6 mg. for 100 gm. rats, and 8.4 mg. for 170 gm. rats. Donaldson's values at both weights are almost exactly twice those of McCarrison, and in light of our experience with rats from Wistar stock, we suspect that his diet was deficient in iodine. It has not been shown that McCarrison's values will hold for rats whose growth has been retarded, as by our diet, but this seems probable. We have not as yet been able to devise a diet that will produce normal growth and at the same time be sufficiently deficient in iodine to produce consistent goiter.

We believe that for comparison of groups whose attained weight does not differ more than 20 gm., comparison on the basis of body weight is sound, but where differences as much as 50 gm. exist, some cognizance of the relatively smaller

normal thyroid size of larger animals must be taken. From McCarrison's data, the thyroid weight, per 100 gm. body weight, of 170 gm. rats, is 6.6 % less than that of 120 gm. rats; or, on our normal value of approximately 12 mg. for the smaller rats; those attaining 160 to 170 gm. weight should give 11.2 mg. The difference is not great enough to invalidate our argument.

The response to dried haddock is, if anything, superior to what one would have predicted from its iodine content. However, both levels of haddock feeding were higher than what we now consider the critical range (viz., 0.3 to 0.8 γ per day), hence we must hesitate to say that its iodine is more adequately utilized than the other foods tested or than iodide, especially since, in this particular experiment, the response to potassium iodide was greater with respect to both thyroid size and dry matter than in the others.

Irish moss, fed at two levels, proved less effective than milk, oysters, or haddock, and less effective than we should have predicted from earlier experiments with iodide. Whether this is a peculiarity of this particular product, or points to a difference in availability of iodine from plant and animal sources, is yet to be determined.

The experiments, taken together, show with excellent agreement that, when fed at similar levels of iodine; milk, oysters and haddock possess antigoitrogenic properties of the same order of magnitude as potassium iodide. Marked advantage from either form of iodine, food or inorganic, when fed at minimal effective levels, does not appear.

SUMMARY

Foods rich in iodine, dried milk, oysters and haddock, and Irish moss, were fed to rats as supplements to a goiter-producing diet.

The antigoitrogenic properties of the milk, oysters and haddock are in proportion to their iodine content, and of the same order of magnitude as those of potassium iodide.

Irish moss, on the other hand, possesses lower antigoitrogenic power than is indicated by its iodine content.

The goiter-preventing properties of milk and oysters are correlated with their iodine content, and not with the improvement in protein quality or mineral and vitamin supply when they are added to a diet containing protein of poor biological quality, low in vitamins, and not adequately balanced as to minerals.

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THE EFFECT OF THE VITAMIN A (CAROTENE) INTAKE ON THE VITAMIN D REQUIREMENT OF RATS IN THE PRODUCTION AND CURE OF RICKETS [1]

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A vast amount of information regarding those factors which influence the physiological processes of ossification has accumulated during the past few years. Attention has been focused primarily upon the calcium and phosphorus content of the diet and upon the presence of the antirachitic substance or substances. It has been conclusively demonstrated that certain variations among these three factors will affect the structure of bone. Little appears to be known concerning the influence of other dietary factors upon the development and cure of rickets, although there are some indications that factors other than those mentioned above may be important in this connection. This is especially true of the vitamin A content of the diet. In fact, the relationship of this dietary factor to the incidence of rickets has already attracted the attention of a few investigators. Rohmer and Dubois ('30) on conducting a series of experiments upon rachitic children receiving a minimum effective dose of vitamin D (300 to 600 rat units of viosterol per day) accompanied by daily administration of 3000 rat units of vitamin A found that such a

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[2] Presented by Florence S. Tabor to the faculty of the Department of Agricultural and Biological Chemistry of the Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

treatment did not modify the action of viosterol in any way. Bacharach, Allchorne and Hazley ('31) studied the effect of adding fresh carrot to a rachitogenic diet and came to the conclusion that the addition of 1 gm. of fresh carrot daily to such a diet did not affect the rate of growth of rats, whether given in the absence or in the presence of vitamin D. Furthermore, these investigators were unable to find any correlation between the severity of rickets and the rate of growth.

Our knowledge of the marked physiological responses produced by vitamin A rich foods led us to believe that vitamin A might have some effect on the vitamin D utilization. Variations in the carotene content of yellow corn as well as in the Ca/P ratio may play a part in the variability of results which have been obtained in different laboratories. Since pure carotene is now available as a source of vitamin A and since viosterol is available as an uncontaminated source of vitamin D, it appeared desirable to study the problem further. Hence the experiments reported in this investigation were carried out for the purpose of determining whether a rachitogenic diet supplemented with different quantities of carotene, as a source of vitamin A, exhibited any influence upon the incidence and cure of rickets. The results obtained were evaluated from both the chemical and osteological findings, with attempts to correlate chemical analyses with radiographic and osteological data.

EXPERIMENTAL

In order to study the problem in question young growing animals of known ancestry were depleted of their body stores of vitamin D by feeding a rachitogenic diet and also by feeding a similar diet after it had been supplemented with different levels of carotene. At the end of the depletion period (twenty-first or twenty-third days) all diets were supplemented with equivalent amounts of vitamin D.

The basal diet used in these studies was the Steenbock yellow corn ration no. 2965 ('25) which consisted (in parts per 100) of yellow corn 76, wheat gluten 20, calcium carbo-

nate 3 and sodium chloride 1. Since it was desirable to maintain a definite dietary composition in order to produce a comparable degree of rickets at all times during the course of the investigation, representative samples of the basal rachitogenic ration were analyzed and found to have the following composition (in parts per 100), protein 24.26, calcium 1.52, phosphorus 0.38.

The various supplements added to the basal diet were: a) Wesson oil, b) a carotene preparation,[3] containing 4200 U. S. P. units[4] of vitamin A per gram, c) a solution of viosterol in olive oil, containing 1 U. S. P. unit of vitamin D per milligram, and d) U. S. P. reference cod liver oil, containing 95 U. S. P. units of vitamin D per gram. An attempt was also made to use a fish-oil concentrate[5] which contained 2,025,000 U. S. P. units of vitamin A per gram as an added source of this vitamin in these studies. The vitamin D content of this product, however, was so great that severe rickets could not be obtained when the desired levels were fed.

Rapidly growing rats 20 to 23 days old and weighing from 39 to 45 gm. each were placed and maintained in individual metal cages, which were provided with raised screen grids. Care was taken to insure uniform distribution of litters and sexes throughout the various groups of experimental animals. During the entire experimental period all animals were kept in a darkened room which was ventilated by means of an electric fan. Distilled water was kept before the animals at

[3] Acknowledgment is gratefully made to the S. M. A. Corporation of Cleveland for the Primatene used in this investigation.

[4] Guaranteed to contain not less than 3000 A. D. M. A. units per gram and converted to U. S. P. units by using conversion factor 1.4.

[5] Acknowledgment is gratefully made to Mr. Harden Taylor of the Atlantic Coast Fisheries, through whose courtesy a fish-oil concentrate of a very high vitamin A potency was obtained. According to Mr. Taylor this product was prepared from a halibut liver oil concentrate by molecular distillation. Previous tests by Dr. R. J. McWalter, University College, London, with the Hilger quartz echelon cell spectrophotometer gave a coefficient of absorption E of 960 at 3280A° through 1 cm. of 1 % solution. The exceedingly high potency ascribed to this product was calculated from data obtained under similar conditions on a concentrate prepared from a sample of U. S. P. reference cod liver oil (3000 units per gram).

all times. All animals were weighed at weekly intervals during the depletion period and a systematic record was made of all changes in body weight and food consumption. For the purpose of dietary treatment, these animals were arranged in experimental groups of eight to sixteen animals per group.

At the end of the depletion period and again at the end of the curative period all animals were x-rayed to determine the state of calcification. At the latter date the animals from each group were sacrificed, the blood was analyzed for calcium and phosphorus, and the long bones of the leg were used for line testing and for bone ash analyses. In the case of the blood it was necessary to pool samples from several animals of the same group, otherwise sufficient amounts were not available for analysis. Duplicate determinations were made in all cases.

To compensate for the lack of sensitivity of the radiographic examination, representative animals from the different experimental groups were killed by bleeding, as controls at various stages of the depletion period, and line tests and bone ash determinations were made of all bones obtained from such animals. In addition, the blood of these animals was used for the determination of calcium and phosphorus.

At the end of the various depletion periods, the remaining animals in all groups were continued on the respective basal diets and, in addition, were given equal amounts of vitamin D of a definite unitage. After these animals had been carried through the desired experimental period, they were killed as described above. The degree of healing or calcium deposition was measured by the x-ray and line test methods and chemical determinations were made for bone ash, blood calcium and blood phosphorus.

The right and left femora and the tibiae and fibulae were used for the line test and bone ash determinations, respectively. These bones were dissected from the animals and freed from all adhering tissue immediately after the animals were killed. The femora were removed for line tests and

photographic records, while the tibiae and fibulae were prepared for ashing by the usual extraction methods, care being exercised to prepare the bones in a uniform manner.

The proximal ends of the femora were examined at the termination of the curative period by the line test technic for evidence of recalcification. The tibiae and fibulae from each leg were wrapped in cheese cloth, dried for 24 hours in an electric oven at a temperature of 100°C., extracted continuously with hot alcohol for 8 hours, and again with ether for the same length of time. The bones were again dried to a constant weight as described above, were weighed in tared crucibles, placed in a muffle furnace and ashed for 4 hours at dull red heat. Crucibles and contents were cooled in a desiccator over night and weighed the following day. The percentage of bone ash was calculated on the basis of the dry extracted bone. Standard deviations for bone ash data were calculated.

Blood samples obtained by cardiac punctures were allowed to stand in the refrigerator over night in order that the serum might exude. The serum was pipetted off and centrifuged to free it from blood cells. An aliquot of the serum was analyzed for calcium by the Clark-Collip ('25) modification of the Kramer-Tisdall ('21) method.[6] The inorganic phosphorus content of the serum was determined by the Youngburg et al. method ('30).

DATA

An outline of the general plan of the investigation is given in table 1. In order to conserve space, summaries of data have been condensed to tabular form and are presented in tables 2 and 3.

The general plan, which was applicable to all groups in each series of experiments, consisted first in developing definite experimental rickets in animals under uniform experimental conditions by: a) Feeding rachitogenic diets to control groups, b) supplementing the basal rachitogenic diet

[6] The mixture was allowed to stand over night for complete precipitation of the calcium oxalate.

with definite quantities of carotene as the source of vitamin A, and c) supplementing the basal rachitogenic diet as well as the diets containing the added amounts of carotene with 5 % of Wesson oil.

It will be observed that the outline presented in table 1 has been divided into two parts, i.e., the depletion period and the curative period. It will also be observed that two sources of vitamin D were used as supplements during the curative

TABLE 1

General outline of the experimental procedure during depletion and curative periods

SERIES	GROUP NO.	DEPLETION PERIOD				CURATIVE PERIOD		
		Number of rats	Days on depletion	Supplements to basal rachitogenic diet		Number of rats	Days on curative	Total Vitamin D intake
				Vitamin A [1]	Wesson oil			
				U.S.P. units	%			*U.S.P. units*
A	1	4	21	..	..	8	18	18[2]
	2	4	21	22	..	8	18	18[3]
	3	4	21	90	..	8	18	18[3]
B	4	5	23	..	..	5	14	14[2]
	5	5	23	22	..	5	14	14[3]
	6	5	23	90	..	5	14	14[3]
C	7	5	23	..	..	5	12	12[2]
	8	5	23	22	..	5	12	12[3]
	9	5	23	90	..	4	12	12[3]
D	10	2	23	..	..	5	14	1[2]
	11	2	23	..	5	7	14	1[3]
	12	2	23	22	5	8	14	1[3]
	13	2	23	90	5	5	14	1[3]
E	14	4	21	..	..	7	14	3.99[4]
	15	4	21	..	5	8	14	3.99[4]
	16	4	21	22	..	8	14	3.99[4]
	17	4	21	22	5	8	14	3.99[4]
	18	4	21	90	..	7	14	3.99[4]
	19	4	21	90	5	8	14	3.99[4]
F	20	6	23	..	5	8	14	3.99[4]
	21	8	23	90	..	8	14	3.99[4]
	22	7	23	90	5	6	14	3.99[4]
G	23	6	21	..	..	11	16	...

[1] U. S. P. units for each gram of diet.

[2] Viosterol in petroleum ether.

[3] Viosterol in Wesson oil.

[4] U. S. P. reference cod liver oil in Wesson oil.

TABLE 2

Summary of the results obtained during the depletion period [1]

SERIES	GROUP NO.	SUPPLEMENTS TO BASAL RACHITOGENIC DIET		AVERAGE WEIGHT		AVERAGE DAILY FOOD INTAKE	AVERAGE DAILY VITAMIN A INTAKE	SERUM MILLIGRAMS PER 100 CC.		AVERAGE PER CENT BONE ASH	STANDARD DEVIATION
		Vitamin A [2]	Wesson oil	Initial	Final			Ca	P		
		U.S.P. units	%	*gm.*	*gm.*	*gm.*	*U.S.P. units*				
A	1	..	..	38.7	62.2	6.1	...	..	5.1	43.38	±0.79
B	4	..	..	42.4	63.6	5.0	...	6.8	..	33.59	±3.02
C	7	..	..	41.2	57.4	4.5	...	..	3.7	33.61	±1.21
D	10	..	..	34.5	55.0	5.5	...	..	Lost	25.80	±1.32
E	14	..	..	55.2	72.7	6.5	...	6.4	5.0	41.07	±2.59
D	11	..	5	44.5	61.0	5.5	...	..	4.8	32.48	±1.47
E	15	..	5	55.7	65.6	5.4	...	6.7	5.7	51.23	±0.23
F	20	..	5	57.6	66.6	5.7	...	6.0	4.8	41.97	±1.88
A	2	22	..	39.7	60.2	5.5	121	..	5.8	44.60	±1.17
B	5	22	..	39.2	61.2	4.9	108	6.2	..	31.14	±1.17
C	8	22	..	41.2	56.4	4.3	95	..	4.1	32.47	±2.43
E	16	22	..	48.0	66.0	5.2	114	5.2	5.8	48.34	±1.42
D	12	22	5	42.0	52.0	4.9	108	..	Lost	30.55	±3.45
E	17	22	5	45.0	63.0	4.8	106	6.8	6.1	43.35	±0.59
A	3	90	..	42.7	61.7	4.3	387	..	4.1	38.76	±0.42
B	6	90	..	44.8	68.8	5.5	495	7.0	..	30.87	±1.44
C	9	90	..	40.4	57.2	5.6	504	..	4.0	32.20	±2.17
E	18	90	..	43.0	61.0	5.2	468	5.9	5.6	43.22	±1.93
F	21	90	..	60.0	77.5	5.9	531	5.6	4.1	42.81	±3.33
D	13	90	5	40.5	54.5	5.0	450	..	5.2	29.30	±3.95
E	19	90	5	48.8	62.5	5.4	486	6.2	5.2	42.61	±1.06
F	22	90	5	54.3	63.9	5.1	459	4.8	5.2	44.04	±3.06
G	23	..	..	37.8	59.6	4.9	...	6.8	..	32.24	±2.88

[1] For number of rats used and length of depletion period see table 1.

[2] U. S. P. units for each gram of diet.

TABLE 3

Summary of the results obtained during the curative period[1]

SERIES	GROUP NO.	SUPPLEMENTS TO BASAL RACHITOGENIC DIET		TOTAL VITAMIN D INTAKE	AVERAGE WEIGHT		AVERAGE DAILY FOOD INTAKE	AVERAGE DAILY VITAMIN A INTAKE	LINE TEST AVERAGE	SERUM MILLIGRAMS PER 100 CC.		AVERAGE PER CENT BONE ASH	STANDARD DEVIATION
		Vitamin A[2]	Wesson oil		Initial	Final				Ca	P		
		U.S.P. units	%	*U.S.P. units*	*gm.*	*gm.*	*gm.*	*U.S.P. units*					
A	1	..	..	18[3]	66.1	74.5	5.2	...	2.5	...	4.2	48.96	±0.31
B	4	..	..	14[3]	59.4	63.8	4.8	...	3.8	6.2	..	32.59	±1.26
C	7	..	..	12[3]	55.0	59.6	4.5	...	2.8	...	4.9	33.97	±2.83
D	10	..	..	1[4]	54.6	49.6	4.0	...	1.0	...	5.2	24.18	±5.01
E	14	..	..	3.99[5]	66.5	66.0	5.0	...	1.0	11.3	6.9	36.36	±3.43
D	11	..	5	1[4]	62.0	68.5	6.1	...	0.7	...	5.9	32.62	±5.87
E	15	..	5	3.99[5]	57.7	58.3	3.9	...	1.3	11.4	6.0	45.63	±1.36
F	20	..	5	3.99[5]	67.3	74.0	6.0	...	1.5	11.4	5.1	43.97	±8.53
A	2	22	..	18[3]	58.5	68.2	4.3	95	1.7	...	3.5	47.96	±1.84
B	5	22	..	14[3]	66.8	73.5	4.9	108	2.5	Lost	..	35.42	±1.40
C	8	22	..	12[3]	56.2	61.8	4.2	92	2.2	...	5.0	33.02	±3.71
E	16	22	..	3.99[5]	67.1	68.1	5.5	121	1.6	10.9	5.3	44.67	±1.59
D	12	22	5	1[4]	65.0	70.8	5.3	117	0.9	...	7.0	32.86	±7.25
E	17	22	5	3.99[5]	64.6	64.6	3.9	86	1.0	11.5	5.6	46.09	±2.37
A	3	90	..	18[3]	60.4	68.5	4.4	396	2.1	...	5.1	39.63	±1.72
B	6	90	..	14[3]	63.8	67.8	4.6	414	2.6	7.0	..	34.03	±4.17
C	9	90	..	12[3]	57.5	64.7	4.5	405	1.5	...	6.1	32.29	±1.85
E	18	90	..	3.99[5]	71.0	76.4	5.6	504	0.7	9.5	6.8	48.34	±3.30
F	21	90	..	3.99[5]	70.4	81.6	6.7	603	2.3	7.4	5.6	42.78	±3.33
D	13	90	5	1[4]	54.8	58.4	3.4	306	0.6	...	6.9	27.65	±8.27
E	19	90	5	3.99[5]	58.5	62.0	4.1	369	0.8	6.9	5.3	42.11	±2.45
F	22	90	5	3.99[5]	68.0	76.1	5.0	450	0.9	6.1	6.1	44.47	±2.40
G	23	..	..	...	41.2	68.3	5.4	...	..	10.3	3.8	27.75	±2.28

[1] For number of rats used and length of curative period see table 1.

[2] U. S. P. units for each gram of diet.

[3] Viosterol in petroleum ether.

[4] Viosterol in Wesson oil.

[5] U. S. P. reference cod liver oil in Wesson oil.

periods; namely, irradiated ergosterol in olive oil and U. S. P. reference cod liver oil. Either Wesson oil solutions or petroleum ether solutions of the vitamin D supplements were used. The data have been likewise summarized with reference to depletion or curative periods and are presented in tables 2 and 3, respectively.

The values for serum calcium and serum phosphorus as given in tables 2 and 3 represent group averages. Owing to limited supplies of blood serum from certain groups of animals, it was not feasible to determine both calcium and phosphorus. Degrees of healing are expressed by line test values in the conventional manner, i.e. [(—) (±) (+) (++) (+++)]. The degree of recalcification which is characterized by an unbroken thin line of darkened granules appearing on the metaphyseal side of the epiphyseal cartilage is expressed by (++), greater or lesser degrees of recalcification being designated accordingly.

In the first series of experiments (series A), three groups of animals (twelve animals per group) were used. The animals of group 1 received the unsupplemented Steenbock rachitogenic diet. The animals of group 2 received the above diet after sufficient carotene (see footnote 3) had been added to furnish 22 U. S. P. units of vitamin A for each gram of food, while the animals of group 3 received a similar diet fortified with carotene to the extent of 90 U. S. P. units of added vitamin A for each gram of food. The diets used in each series of experiments of this type were made up in quantities sufficient to complete the experiments, stored in air-tight containers, and maintained at a low temperature in an electric refrigerator. After the animals comprising this series had been on the rachitogenic diets for 21 days, four animals from each group were killed and used as controls. The remaining eight animals of each group received, in addition to the respective basal diets, 1 U. S. P. unit of vitamin D per day in the form of viosterol. The feeding of this vitamin D supplement was continued for a period of 18 days, at the end of which time the animals were killed, line tested, etc.

The animals in the second and third series of experiments (B and C) were depleted for a period of 23 days, since it appeared highly desirable to obtain a more marked degree of rickets than had been observed in series A. The curative periods were decreased to 14 and 12 days, respectively, since, in the first series (series A), recalcification was so advanced in all groups that sharp differentiation between groups was difficult.

Further variations were introduced in series D, through the introduction of a definite amount of fat in each of the above diets. In this series four groups of animals were used. The animals of group 10 received the usual rachitogenic diet, while group 11 received this basal diet after 5 % of Wesson oil had been added to it. Groups 12 and 13 received the diet containing 5 % of Wesson oil, which also contained 22 and 90 units of vitamin A per gram, respectively. The vitamin D supplement used in this series was viosterol which was made up to such a volume (in Wesson oil) that 1 cc. was equivalent to 1 U. S. P. unit. This supplement was fed in special receptacles on the first, fifth and tenth days in 0.4 cc., 0.3 cc. and 0.3 cc. portions.

In order to study the effect of the presence of fat in the diet on the vitamin A-vitamin D relationship, a fifth series of experiments was carried out. This series of experiments (series E) consisted of six groups of animals. In the beginning each group was composed of twelve animals, but unfortunately a number of these animals died during the course of the curative period. The depletion period for this series was 21 days, since radiographic examination at that time revealed that all animals showed a marked degree of rickets. The animals of this series were arranged in groups in such a manner as to make possible a comparison of the responses of those receiving diets supplemented with 5 % of Wesson oil with the responses made by other groups receiving the rachitogenic diet containing no added fat. The vitamin D supplement for this series was a solution of U. S. P. reference cod liver oil containing 95 U. S. P. units of vitamin

D per gram. This vitamin D supplement was fed in separate containers in 0.5 cc. portions on the first, fifth and tenth days. This amount of oil was equivalent to 3.99 U. S. P. units.

Series F constituted a repetition of a portion of the feeding trials of series E, i.e., of groups 15, 18 and 19. In this series the depletion period was extended to 23 days because x-ray examinations on the twenty-first day revealed that the degree of rickets manifested at that time was somewhat less than was desirable.

The animals comprising series G were used as negative controls. These animals were maintained for 37 days on the basal rachitogenic diet without additional supplement. At the end of this period, the animals were examined to determine the state of ossification, using the various criteria described elsewhere.

DISCUSSION

These experiments were designed primarily for the purpose of determining whether vitamin A exerts any influence upon vitamin D utilization by the rat, the vitamin A being fed at different levels, while the amount of vitamin D remained constant. While agreement of opinion regarding the cause and cure of rickets is still lacking, four methods of evaluating the results in the incidence and cure of this disorder have been quite well established; namely, x-ray, line test, bone ash and chemical analyses of the blood for inorganic constituents, especially calcium and phosphorus. These criteria have been used in an attempt to establish the etiological interdependence of vitamins A and D in the production and cure of rickets. In many instances lack of correlation between these criteria has been strikingly demonstrated.

The effect of adding carotene as the source of vitamin A to the basal rachitogenic diet may be observed from the results obtained in series A (table 2). Mild rickets was manifested by animals comprising the control group (group 1) as well as by those animals of group 2 which received the rachitogenic diet supplemented by 22 units of vitamin A.

Severe rickets was very apparent among those animals of group 3 which received the basal rachitogenic diet supplemented by 90 units of vitamin A. Similar observations were made among comparable groups of animals in series B and C. The marked similarity of results obtained from the several groups of animals comprising series D indicates that when Wesson oil was added to the diet there was no appreciable effect on the severity of rickets produced, in spite of the fact that the total gain in weight made by the animals on the low fat diet (group 10) exceeded that of all other groups of the series. A discrepancy appears in the results obtained from all groups of series E. Rickets was evident among the animals of the control group (group 14), while no indication of this disorder appeared in group 15 and only very mild rickets was observed in groups 16, 17, 18 and 19 of this series. Examination of the data does not disclose that the animals had either lost weight or were refusing food but, on the contrary, all groups had shown appreciable gain in body weight. In order to verify the results obtained in the above series, this series (series E) was repeated using three groups of animals (series F). But here again the animals of the control group (group 20) showed evidence of definite rickets, whereas those of groups 21 and 22 manifested no greater severity than had previously been shown by comparable groups in the preceding series.

In these studies x-ray technic was found to be less accurate than the osteological technic in determining the degree of rickets. It was found, however, to be sufficiently accurate to indicate that the majority of animals receiving diets supplemented with 90 units of vitamin A per gram of food manifested a more marked degree of rickets than did the control animals or those animals receiving lower levels of vitamin A.

A second criterion for estimating the intensity of rickets was the variation in bone ash values. An inspection of these data shows a serious lack of consistency. In the case of series A high bone ash values were obtained for the animals of groups 1 and 2 (43.38 and 44.60 %) and decidedly lower

figures were obtained for those of group 3 (38.76 %). Similar irregularities, although less marked, were obtained in series B and C.

There appeared to be no appreciable differences in the average bone ash values among the groups of animals comprising these two series. The bone ash values obtained in series D were noticeably lower than those which had been obtained in any of the preceding experiments. It should be noted, however, that this constituted a preliminary series in which an insufficient number of animals was used. The bone ash value for the control group (group 10) was the lowest obtained (25.80 %), although it was noted that the animals in this group made a greater increase in body weight than did the animals of the succeeding groups. It is possible to explain the low figure mentioned above on the assumption that rate of growth is recognized as a predisposing factor in the etiology of rickets. The bone ash values for the animals of the remaining groups in the series were 32.48, 30.55 and 29.30 %, respectively.

Since the data obtained in the experiment of series D indicated some tendency toward differences in bone ash values when 5 % of Wesson oil was used as an added supplement to the rachitogenic diet, it seemed advisable to repeat the experiments of series D using a larger group of animals. The data obtained in this series (series E) disclose surprisingly inconsistent results. The animals of group 14 gave an average bone ash value of 41.07 %, yet x-ray examination had indicated severe rickets in all animals of the group. The animals in group 15 manifested little or no evidence of rickets and the average bone ash value for the group was 51.23 %. In group 16 two animals gave no indication of rickets when observed radiographically, two animals showed mild rickets, and the average bone ash value for all animals of this group was 48.34 %. The bone ash values obtained for groups 17, 18 and 19 were 43.35, 43.22 and 42.61 %, respectively.

In an attempt to clarify the discrepancies just described, another experiment consisting of three groups of animals

was carried out. The three groups of animals composing this series (series F) received the same dietary treatment as those of groups 15, 18 and 19. The animals of group 20 of this series exhibited definite rickets in all cases, although the average bone ash value remained at 41.97 % or considerably lower than the value obtained in group 15 of the preceding series. Values for groups 21 and 22 were in agreement with those obtained in series E, in that there was no appreciable lowering of bone ash values although rickets was manifest to a certain degree in all animals of the series.

Examination of the data in table 2 discloses a wide variation in bone ash values. It is apparent that definite correlation does not always exist between severity of rickets (as measured by line tests) and bone ash determinations, although duplicate determinations, when made on the bones of individual animals, agree consistently. A factor which may have some bearing on the above discrepancies is the age of the test animals. Bone ash determinations on the extracted bones from normal animals of various ages gave the following values: 18 months, 63.20 % $\pm$ 1.16 (S.D.)[1]; 12 to 15 months old, 56.03 % $\pm$ 1.36 (S.D.); 40 to 50 days old, 50.35 % $\pm$ 2.01 (S.D.); and 30 to 40 days old, 48.34 % $\pm$ 1.64 (S.D.). The value obtained for negative controls, animals at the end of a 37-day depletion period, was 27.80 % $\pm$ 2.28 (S.D.).

Blood phosphorus values obtained in series A, C and D disclosed no significant differences. Blood calcium values obtained in series B were somewhat lower than normal values, although there was not a very great difference between individual groups of the series. The average value for the animals of the control group (group 4) was 6.8 mg. of calcium per 100 cc. of serum, while the values for the other two groups were 6.2 and 7.0 mg. per 100 cc. of serum respectively.

In the case of the animals comprising the various groups of series E and F, both blood calcium and blood phosphorus values were determined. An examination of the data in table 2 shows that the product of these values for all groups,

[1] S.D. (standard deviation).

except group 17, was 30 or less. Kramer, Shear and Siegel ('31) have reported that active rickets is present when this product falls below 30. The radiographic and bone ash values for these experiments, however, do not warrant such a conclusion. The addition of 22 and 90 units of vitamin A tended to lower blood calcium and blood phosphorus values, although the differences between groups were slight. Attention is called to the fact that bone ash values remained consistently high in spite of the appreciable lowering of blood calcium and blood phosphorus. In other words, there does not seem to be a definite correlation between bone ash, blood calcium and blood phosphorus values as obtained in these series of experiments during the depletion periods.

The influence of vitamin A on the vitamin D utilization and, consequently, on the cure of rickets may be deduced by likewise considering data obtained in the curative periods. An analysis of the line test data as summarized in table 3 shows that, in general, lower values for average line test healings were observed in all groups receiving vitamin A supplements than were observed among animals of the control group. The lowest values were observed among those groups receiving the basal ration supplemented with 90 units of vitamin A and 5 % of Wesson oil simultaneously. There appears to be less correlation between bone ash values and line test values at this time than there was between radiogram and bone ash values at the end of the depletion periods.

Further examination of the data discloses that wide variation in bone ash values occurred among the various groups of test animals. In series A there was no definite increase in bone ash in any of the groups over and above that observed at the close of the depletion period. Group 3, with the lowest value of 39.63 %, gave a relatively high line test average (2.1), while group 2 with a bone ash value of 47.96 % gave a line test average of only 1.7. More striking discrepancies appear among the bone ash data of series B and C; yet all of these groups gave high line test averages. Relatively low bone ash values were found for all groups of animals com-

prising series D. The lowest bone ash value was given by the control group (group 10); however, this group gave the highest line test value.

Inconsistencies in bone ash values are further emphasized in those groups comprising series E. The control group (group 14) gave a lower value for bone ash at the end of the curative period than was observed at the end of the depletion period. Similar observations are noted among the animals of groups 15 and 16. Groups 17 and 18 manifested a marked increase in bone ash values for the curative period, while group 19 showed little difference between bone ash values at the end of the depletion period and at the end of the curative period. The results obtained in series F were very comparable to those obtained in series E. On the basis of these bone ash data only indefinite information is afforded relative to the degree of recalcification. This opinion has been expressed by other investigators (Adams and McCollum, '28).

Since there appeared to be a definite lack of correlation between bone ash values and the corresponding line test averages, an attempt was made to determine the range in line test values which would result in a measurable difference in the percentage of bone ash. For this purpose the tibiae (fibulae attached) from both legs of four animals which had shown a 4(+) line test healing were removed and bone ash values were determined according to the technic previously described. The average bone ash value obtained for this group of animals was 38.5 % $\pm$ 3.6 (S.D.). This same procedure was repeated using six animals, which gave a 1(+) line test healing. The average bone ash value for this group was 33.1 % $\pm$ 2.47 (S.D.). In other words, a difference between a 4(+) and a 1(+) in line test values was necessary to show a difference of approximately 5 % in bone ash values. It appears, therefore, that small differences in the degree of recalcification cannot be measured quantitatively by the bone ash technic.

Variations in blood calcium and blood phosphorus were obtained among those series of animals which had received vitamin D from different sources as a supplement to the rachitogenic diets. One U. S. P. unit of vitamin D daily (viosterol in petroleum ether) was the supplement used in series A, B and C. In general, it may be pointed out that very small increases in the values over those found at the end of the depletion period were manifested. Blood phosphorus values were determined for series A and C. In series A this value increased in group 3, while for groups 1 and 2 the value was lower than at the end of the depletion period. In series C all groups manifested an increase in blood phosphorus; group 9 (receiving 90 units of vitamin A per gram of food intake) gave the highest value. Blood calcium was determined for series B. The increase in this element over that observed at the end of the depletion period was very slight. In series D the supplement was 1 U. S. P. unit of vitamin D (viosterol in Wesson oil). In this series an increase in the blood phosphorus was observed among all groups of animals, the values ranging from 5.2 to 7.0 mg. of phosphorus per 100 cc. of serum.

Blood calcium and blood phosphorus values were determined in series E and F, and here some interesting observations were made. While marked increase appeared in the calcium values above those observed at the end of the depletion periods, there was no great increase in the blood phosphorus values of either series. No difference in the blood calcium value for the control group (group 14) and the group which received the rachitogenic diet plus the Wesson oil (group 15) was apparent. A similar value (11.5 mg. of Ca per 100 cc. of serum) was given by group 17, while lower values were obtained in groups 16, 18 and 19. These values ranged from 10.9 to 6.9 mg. of Ca per 100 cc. of serum.

There appeared to be but slight differences in the blood phosphorus values among the various groups of these series, the values ranging from 6.9 to 5.3 mg. per 100 cc. of serum.

The values for series F were similar to those for corresponding groups in series E. In this series a reference cod liver oil, in an amount equivalent to 3.99 U. S. P. units of vitamin D, was fed as the supplement. The average blood calcium and blood phosphorus values for groups of control animals on this supplement after a 10-day curative period was found to give 11.4 and 5.2 mg. per 100 cc. of serum, respectively.

An examination of the values obtained by the chemical analyses of the blood serum shows a lack of correlation between calcium and phosphorus values, line test values, and bone ash values. Differences of opinion and conflicting results concerning such findings appear in the literature. It has been observed by Kramer, Shear and Siegel ('31) that recalcification in rachitic bones is preceded by a rise in the blood calcium and phosphorus. Hess et al. ('30) have observed that there is a lack of correlation between healing rickets and the inorganic phosphorus of the blood and that, while in general a lowering of phosphorus takes place, this is not necessarily an essential feature of rickets.

The age of the test animal may have some bearing on the diversity of results. Analyses of blood for inorganic calcium and phosphorus from normal animals of various ages gave the following values in milligrams per 100 cc. of serum: 3 to 5 weeks—calcium 7.6, phosphorus 8.7; 90 days—calcium 9.8, phosphorus 6.6; 17 months—calcium 9.6, phosphorus 6.3; 20 months—calcium 10.9, phosphorus 6.3.

SUMMARY

The production of rickets in the growing rat receiving a rachitogenic diet supplemented and unsupplemented with carotene as the source of vitamin A has been studied by means of x-ray examination, line test, bone ash determinations, and by an analysis of the blood serum for calcium and phosphorus. The subsequent recalcification, when corresponding animals received such diets supplemented with vitamin D from two sources, has been investigated in a similar manner.

The manifestations of the interrelationship of vitamin A and vitamin D in the etiology of rickets depend no doubt upon several factors, among which may be mentioned 1) past ancestral and nutritional history, 2) the age of the animals, and 3) individual variation among animals of the same age. It may be observed that the values found for the percentage of bone ash for normal animals of different ages varies with age. There is also variation in the blood calcium and blood phosphorus among normal animals of different ages. It is, therefore, highly desirable that all experimental animals used in a study of this nature be from the same genetic source and be carefully standardized according to age.

The efficient conversion of carotene to vitamin A undoubtedly varies among individual animals. Consequently, a detailed study of the problem necessitates the use of a natural vitamin A concentrate free from vitamin D in order to insure a clearer understanding of the vitamin A-vitamin D relationship. Until it is possible to procure such a supplement, this interrelationship cannot be readily demonstrated.

The following observations were made during the course of the experiment:

Rachitogenic diets containing 90 U. S. P. units of vitamin A per gram of diet seemed to produce, during the depletion period, an increase in the severity of rickets among growing rats over and above that produced by a similar diet which did not contain added vitamin A supplement.

The radiographic examination at the end of the depletion period was a more reliable index of the intensity of rickets than was the percentage of bone ash.

Evidences of satisfactory correlation between the percentage of bone ash and corresponding radiograms were not obtained.

Inconsistencies between the calcium and phosphorus content of the blood serum and bone ash values were also apparent.

When equivalent unitages of vitamin D were administered, rachitogenic diets containing 90 U. S. P. units of vitamin A

per gram, with and without 5 % of Wesson oil, appeared to retard recalcification (as measured by line test technic) to a greater extent than was observed when similar diets which did not contain any vitamin A supplement were fed.

Data obtained as a result of bone ash determinations and blood serum analyses did not appear to be in agreement with line test data.

From the results obtained in this investigation the line test data appeared to be the most reliable criteria of recalcification.

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ON THE EFFECTIVENESS OF MALT AMYLASE ON THE GASTRIC DIGESTION OF STARCHES

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This work was undertaken to determine the in vivo effectiveness of malt amylase on the digestion of starches. Our results bear more directly on the subject of the effectiveness of the enzyme on the gastric digestion of starches; but in addition the results show that a portion of the enzyme passes into the intestine before it is inactivated by the acid of the stomach and may aid the intestinal digestion of starches. Although salivary amylase has also been studied, most of the investigation has involved the use of malt amylase which was chosen because it is inactivated at a lower pH (pH 2.5; optimum pH 4.5) (Sherman, Thomas and Baldwin, '19) than salivary amylase (pH 4.5; optimum 6.6) and, hence, should theoretically be more effective for therapeutic use in deficiency of pancreatic amylase.

The chief specific questions we desired to answer were: a) How much digestion of starch actually occurs in the stomach before salivary and malt amylase is inactivated? In regard to malt amylase this question has been considered by others on the basis of a priori evidence and in direct experimentation. In regard to salivary amylase the observations of Bergeim ('26) on man are generally accepted. We desired more crucial experimental evidence, and to extend Bergeim's observations, using malt amylase as well as salivary amylase. b) How much of orally administered enzyme passes into the intestine in an active form under various conditions, such as

when the enzyme is mixed with the meal or when it is taken after a meal?

The investigation has been conducted on dogs and human subjects. The dog has served us especially because it permits the performance of crucial analytical studies, has no ptyalin in its saliva, and, hence, yields data on which to interpret the results of perhaps less crucial experiments on man.

CHEMICAL METHODS

Substrate. As a substrate we have used both 5 % cornstarch paste and a 7½ % farina (wheat) cereal paste as a test meal. The cereal was cooked 20 minutes to insure a homogeneous mixture. In certain experiments on human subjects a mixed meal was used.

Malt amylase. After assaying several commercial products of malt extract, a commercial preparation[1], which proved to be quite uniform in activity, was chosen. One gram of this preparation, under optimum conditions of substrate and enzyme concentration and at a pH of 6.8, assayed 0.3050 Willstätter et al. ('23) amylase units. Under similar conditions but at a pH of 4.5, 1 gm. assayed 0.405 Willstätter amylase units and converted 2.0 times its weight of starch to water soluble reducing substances in 15 minutes at 37°C. Suitable control tests on the amylase content of the extract were performed (table 1) every time an experiment was made, the necessary correction for the reducing substances in the amylase preparation being made. The desired amount (vide infra) of the malt extract preparation in solution in water or milk was either mixed thoroughly with the substrate or drunk with the stomach full of food. Malt extract was chosen in preference to takadiastase because the former has a higher saccharogenic value.

The maximum amount of reducing substances calculated as maltose that could be obtained from 7.5 gm. of farina was 4.5 gm.; while 5 gm. of cornstarch (commercial) yielded 3.0 gm. of maltose. The per cent of digestion was determined on the basis of these figures which were repeatedly checked. Sufficient malt diastase was added to the test meals to give complete digestion under optimum conditions.

[1] Ovaltine.

Determination of dilution of substrate with gastric secretion. It was necessary, in order to estimate the reducing sugars formed and present, as well as active enzyme, in the various samples obtained from the stomach, to know the degree of dilution of the substrate with gastric juice. This was

TABLE 1

Showing the digestion of starch with and without malt amylase when introduced in an isolated pouch of the entire stomach

OBSERVATIONS MADE	CONTROL EXPERIMENTS: NO AMYLASE ADDED			AMYLASE OR MALT EXTRACT ADDED		
	In vitro sample[1]	Samples taken from the stomach		In vitro sample[1]	Samples taken from the stomach	
		30 minutes	60 minutes		30 minutes	60 minutes
Dilution	1.00	0.88	0.72	1.00	0.86	0.82
pH	6.5	2.8	2.3	6.3	2.6	2.0
Milligrams per cent reducing substances	250	256	283	445	1043	1097
Milligrams per cent reducing substances after 1 hour of inactivation[2]	247	270	275	1338	1209	1230
Per cent digestion: Reducing substances found in gastric contents	8.3[1]	8.5	9.4	14.8[1]	34.9	36.5
Reducing substances found after incubating gastric contents for 1 hour[2]	8.2[1]	9.0	9.2	44.6[1]	40.2	41.0

The figures gives the average of three determinations on each of three dogs. Two hundred cubic centimeters of 5 per cent cornstarch continuously perfused through the stomach for 1 hour, 1 mg. of histamine being injected at the beginning of the experiment to stimulate gastric secretion. The stomach was actually perfused for 2 hours, the 1-hour figures being the more significant because of the high acidity at 1 hour prevented much further digestion.

[1] A control sample to determine the amount of reducing substance in test meal. This sample was not introduced into the stomach. Such a control test was made in the course of every test performed in this investigation.

[2] This was done to detect the presence or absence of active amylase.

accomplished by adding phenol red dye to the substrate in a known amount (2 mg. %) and determining its dilution as given below.

Removal of non-sugar reducing substances from the samples. It was found that the gastric secretions, as obtained under the conditions of our experiments, contained non-sugar reducing

substances, or took up some of the iodine which was the agent employed in our assay for maltose. This complicating factor was eliminated by adding an equal amount of a precipitating mixture consisting of equal parts of 10 % sodium tungstate and 1.3 N. H_2SO_4. Control determinations on such filtrates of gastric washings showed them to be free from reducing substances. Known quantities of maltose added to gastric washings, which were then treated by this method were recovered quantitatively, establishing the accuracy of the method.

The determination. It is imperative that the extent of starch hydrolysis be measured by a method that is reliable over a fairly wide range of values. We have investigated a number of the more commonly used methods for determining the reducing sugars resulting from the hydrolysis of starch (Schmidt, Greengard and Ivy, '34) and selected the method of Willstätter, Waldschmidt-Leitz and Hesse ('23) with certain modifications, as being best suited to our needs.

The routine for determining the maltose produced and the activity of the enzyme in a sample was as follows:

Twenty cubic centimeters of the gastric or duodenal collection is divided into two samples, A and B, of 10 cc. each. Duplicate analyses were made in the canine experiments, but could not be made in the human tests.

Sample A is added immediately to a test tube containing an equal volume of previously mixed 1:1 10 % sodium tungstate — 1.3 N. H_2SO_4 which has stood for 5 or 10 minutes. This procedure insures a more complete precipitation of the non-sugar reducing substances and insoluble colloidal starch. The contents of the tube are intimately mixed and the tube is then set aside while the remaining collections are accumulated for analysis.

Sample B is placed in a constant temperature bath (37°) and allowed to incubate for 1 hour, after which it is inactivated by treating with the precipitating mixture by the same technic used with sample A. Sample B is so treated to ascertain the amount of active diastase that passed through the stomach into the duodenum, or the amount of active enzyme in the gastric sample.

Both series of tubes (A and B) are then centrifugalized and a 5 cc. portion of the supernatent fluid from each is transferred into 250 cc. Erlenmeyer flasks. These 5 cc. portions are used for the sugar determinations. Other 5 cc. portions of the 'A' series are pipetted into test tubes for the determination of phenol red concentration. To each of the latter is added 1 cc.

of concentrated NaOH The resulting purplish-red color is compared in a colorimeter against an identically treated portion of the original cereal test meal which serves as the standard. Diluting the standard to one-fourth or one-half its original concentration gives a standard that matches the unknown samples closely and within the range of optimum accuracy. The values expressed in our tables under the heading 'dilution' represent the percentages of dye in the diluted gastric samples as compared to the original concentration of the test meal when introduced into the stomach.

To the fluid in each Erlenmeyer flask are added 10 cc. N/10 KI_3 and the mixture is then made alkaline by slowly adding, with shaking, N/8 NaOH. About 50 cc. of the alkali are required to convert the iodine to sodium hypoiodite which is colorless; however, due to the presence of the phenol red dye the contents of the flask appear pink when this point is reached. The flask is then allowed to stand 15 minutes after which 2 cc. of 10 % H_2SO_4 are added. The sample is then titrated to a colorless (yellowish) end point with N/20 $Na_2S_2O_4$. One cubic centimeter of N/10 KI_3 is equivalent to 17.15 mg. of maltose so long as the volume of iodine taken up is between 0.5 and 3.5 cc. Above a titration value of 3.5 cc. of KI_3 a straight line relationship no longer exists for the KI_3-maltose ratio. In order that the existing curvilinear relationship be taken into consideration, we have plotted a curve based upon the iodine taken up by known amounts of maltose varying from 10 to 100 mg. Titration values on routine determinations are referred to this curve for maltose equivalents.

The reducing sugar is expressed in terms of milligram maltose per 100 cc. of the original substrate by the following calculation:

$$\frac{\text{Mg. maltose in 5 cc.} \times 40^{a}}{\text{Per cent of original concentration. (from phenol red determination)}} = \text{Mg. per cent maltose}$$

It has been pointed out that 7.5 gm. of farina yielded 4.5 gm. of maltose. The value mg. per cent maltose is calculated to per cent digestion by dividing by 4500 mg. The difference between the values in samples A and B indicates the presence or

[a] Five cubic centimeters, taken for sugar determination, multiplied by four equals the amount in the 20 cc. of treated gastric or duodenal sample. This value represents the amount of reducing sugar in 10 cc. of the cereal substrate, therefore, the amount in 5 cc. times four times ten equals milligram maltose in 100 cc. of the diluted sample. Dividing by the concentration of the diluted sample gives the milligram maltose per 100 cc. in terms of the original concentration.

absence of active amylase. This difference may be expressed in terms of Willstätter units by substituting the appropriate values into the formula $K = \frac{1}{t} \log \frac{a}{a-x}$ (Willstätter, et al., '23).

PHYSIOLOGICAL METHODS

In dogs

Digestion of starch in the stomach and the degree of activity of the amylase administered was studied by three different physiological techniques, which supplied crucial data.

A. *Dogs with a pouch of the entire stomach.* Three dogs with a pouch of the entire stomach were employed. In these under asceptic conditions the stomach was isolated from the alimentary tract. The cardiac end of the stomach was closed and the pyloric end opened to the outside by a fistula, the integrity of the alimentary tract being restored by an esophagoduodenal anastomosis (Lim, Ivy and McCarthy, '25). These animals, of course, remain in excellent condition for years. With this preparation it was possible to place the substrate with or without malt extract into the stomach within a period of 5 minutes and study digestion without possible contamination with intestinal secretions. Further, the gastric glands of the pouch could be stimulated with histamine, so as to follow the effect of pH on the course of the digestion. In these animals 200 cc. of 5 % starch paste with or without enzyme was placed in the stomach and histamine (1 mg.) was injected. Samples of the substrate were removed at ½-hour intervals from maltose and enzyme assay.

B. *Dogs with a complete duodenal fistula, exclusion of bile and pancreatic juice, and jejunostomy.* Three dogs were prepared as follows: The pancreas was separated from the duodenum to exclude pancreatic secretion. The common bile duct was tied and cut and the gall bladder anastomosed to the ileum, thus excluding bile from the duodenum. This was done at a preliminary operation. At a second operation the duodenum was sectioned 3 inches below the pyloric sphincter, and the distal end was closed and the proximal end converted into a fistula. A jejunostomy was then performed, through which the animal was fed by a technic previously used in this laboratory (Scott and Ivy, '31). The farina meal with or without enzyme was either fed by mouth or introduced via gastrostomy, from 3 to 5 minutes being required to get the meal into the stomach. In this preparation the entire gastric discharge was collected from the duodenal fistula. Saliva, of

course, was swallowed but it is well known that dog's saliva contains only traces of diastase at the most. A small amount of duodenal secretion was probably present in the chyme collected from the duodenal fistula but no pancreatic juice and bile. We, thus, had an almost perfect test animal for these studies.

C. *Dogs prepared as in B, having a gastric fistula, but with an incomplete duodenal fistula through which duodenal chyme was collected by a catheter.* Four such animals were prepared because it is easier to preserve excellent health in them. By means of the phenol red determinations it was shown in each experiment on these animals that we were able to recover through the catheter (24 French) from 40 to 80 % of the chyme emptied from the stomach.

Human subjects

Graduate medical students were used as subjects. The experimental methods will be given later. Determinations of reducing sugar, active amylase present in gastric contents, dilution and acidity were made on the gastric samples. In addition, tests to correlate gastric emptying time and acidity were made.

RESULTS

Total gastric pouch dogs. Six tests were made on each of the three dogs. The introduction of 5 % starch paste without malt extract served as a control for the introduction of the starch paste with malt extract (0.35 gm. desiccated malt extract). The difference between the reducing sugar values served as an index of the extent of enzyme action.

The entire data on starch digestion in the stomach pouches are too bulky to submit; yet they were so consistent, even between different dogs, as to permit the presentation of averages. This is done in table 1. Referring to the table, it is to be noted that the acidity increased and that dilution resulted. The addition of malt extract increased the per cent of digestion of starch, the average at 1 hour being 36.5 %. Ninety-five per cent of this digestion occurred within the first 30 minutes, or during the time the pH of the gastric contents changed from a pH of 6.3 to 2.6. This is due to the fact that the amylase in the malt extract we used is inhibited when the pH falls below 4.0 and inactivated at pH 2.5.

Complete duodenostomy dogs. Both the cornstarch and cereal test meals were used. The cornstarch meal was soon discarded because it was liquefied and evacuated from the stomach so rapidly. At 1 hour, when the stomach was about empty, 60 % of the starch had been digested and very little of the enzyme had been destroyed. The cereal meal (amount varied according to the size of the dog) was not evacuated

TABLE 2

SAMPLE	VOLUME COLLECTED	VOLUME COLLECTED CORRECTED FOR DILUTION	ACID			DILUTION	MILLIGRAM PER CENT MALTOSE		GRAMS MALTOSE RECOVERED	PER CENT DIGESTION	
			pH	Free Cl. U.	Total Cl. U.		At recovery	After incubation to determine active enzyme present		At recovery	After incubation
Dog 3. 'Starch digestion' in complete duodenostomy dogs. 400 cc. 7½ % farina cereal. No malt extract added. Recovered 265 cc. (corrected volume) in 60 minutes. Average of three determinations											
15 minutes	90	78	4.9	5.0	12.5	0.87	408	415	0.32	9.1	9.2
30 minutes	110	90	4.1	10.0	15.0	0.82	420	490	0.38	9.3	10.9
45 minutes	75	55	3.7	12.0	20.0	0.73	515	520	0.28	11.4	11.5
60 minutes	60	42	3.2	15.0	25.0	0.70	556	572	0.23	12.4	12.7
Duodenal collection		265							1.21		
Left in stomach		135							0.74		
Total		400							1.95	10.8	
Dog 3. Effect of malt amylase on starch digestion. 400 cc. 7½ % farina cereal containing 0.70 gm. malt extract. Recovered 316 cc. (corrected volume) in 60 minutes. Average of three determinations											
Control[1]			6.8			1.00	582	1970		12.9	43.8
15 minutes	111	99	5.2	5.0	10.0	0.89	1470	1837	1.45	32.7	40.8
30 minutes	160	136	4.8	6.5	12.5	0.85	1744	2210	2.37	38.8	49.2
45 minutes	85	66	4.4	7.5	15.0	0.78	2260	2351	1.49	50.3	52.3
60 minutes	20	15	3.6	10.0	17.5	0.74	2591	2502	0.39	57.6	55.7
Duodenal collection		316							5.70		
Left in stomach		84							2.18		
Total		400							7.88	43.8	

[1] Not introduced into stomach; in vitro test.

so rapidly, but the most variable factor observed in these experiments was the rate of emptying of the stomach. The individual experiments were not continued until the stomach was completely empty, but until the pH fell to a point at which the enzyme could no longer act. This generally in these dogs required 1 to 1½ hours. The maltose remaining in the stomach could then be easily removed via the gastric fistula, assayed, and the total maltose produced by the amylase calculated.

The results of a typical experiment on one dog is shown in table 2. For comparative purposes only the data obtained

TABLE 3

Summary of data on three complete duodenostomy dogs

DOG NO.	COLLECTED FROM DUODENUM		VOLUME LEFT IN STOMACH (CORRECTED)	pH CHANGE	POTENTIAL MALTOSE INTRODUCED IN FORM OF STARCH (GRAMS)	MALTOSE RECOVERED (GRAMS)			PER CENT OF STARCH CONVERTED TO MALTOSE[2]	PER CENT OF ACTIVE ENZYME RECOVERED IN INTESTINE
	Volume in 1 hour[1]	Corrected volume (dil. × vol.)				From duodenum	Stomach	Total		
1	360	340	60	7.0–5.2	18.0	7.1	1.6	8.7	48.3	85.0
2	375	331	69	7.0–4.8	18.0	9.8	2.1	11.9	66.0	82.7
3	376	316	84	6.8–3.6	18.0	5.7	2.2	7.9	43.8	79.0

Average of all tests. 400 cc. 7½ % farina cereal containing 0.7 gm. malt extract.

[1] One hour arbitrarily chosen to compare results.

[2] This value represents total conversion of starch to reducing sugars during 1 hour in vivo. It differs from 'per cent digestion,' because the latter represents the condition present at the time the sample was taken and can be used for comparative purposes only.

during the first 60 minutes of the test are given. All the data are summarized in table 3.

It was noted that the average emptying time of the cereal meal without enzyme was approximately 80 minutes, whereas with the enzyme the emptying time averaged a little more than 60 minutes. This was obviously due to the liquefying action of the enzyme.

The amount of digestion of the cereal without added enzyme varied from 5 to 12 %, with the added enzyme from 30 to 65 %, the average being 52 %. The higher percentages of digestion,

of course, occurred in those experiments in which the pH of the gastric contents fell more slowly. From 79.0 % to 85.0 %, average 82.2 % of the malt amylase passed into the duodenum without being inactivated. The per cent of digestion was greater in these than in the gastric pouch experiments, because of the higher acidity obtained by histamine injection in the latter.

Two experiments were performed in which the subject was permitted to ingest the cereal meal and then a known quantity of a solution of malt extract in water was drunk by the dog. The discharge from the duodenal fistula was collected and analyzed for amylase to determine the amount that passed. It was found that approximately 75 % of the enzyme solution drunk was passed into the duodenum within 10 minutes; the remainder mixed with the gastric contents. (See Ivy, '18, for evacuation of fluids with food in the stomach.) In these instances the gastric contents showed from 16 to 20 % digestion of the test meal at the end of 1 hour. These results, when compared to the above results, show that the quantity of enzyme that remained in the stomach was not adequate for the digestion of the starch and that when it is desired to have active amylase pass through the stomach rapidly the enzyme should be taken after a meal.

Duodenal and gastric fistula dogs, the duodenal chyme being collected in part by a catheter. In the preceding experiments the dogs were so prepared as to obtain unequivocally the total discharge from the stomach. The four dogs used in the following experiments were easily maintained in excellent health for months. Although the total gastric discharge could not be collected through the catheter (24 French) in the duodenum, from 40 to 80 % of the discharge could be collected and the total discharge calculated from the phenol red determinations. The gastric fistula rendered the determination of the emptying time of the stomach very accurate. Such dogs as a rule do not empty their stomach as rapidly as a dog with a complete duodenal fistula. The enterogastric reflex does not operate well in the latter animals.

The results obtained compare very favorably with the results on the complete duodenostomy dogs. The effect of malt extract on gastric emptying was the same. The total amount of gastric starch digestion was somewhat less on the average varying from 30 to 40 %. This was due to the fact that the acidity of the gastric contents in these dogs rose more rapidly as a rule. However, 87.5 % of active amylase passed into the duodenum. The average results on the dog which showed the least digestion due to a more rapid rise in acidity are shown in table 4.

TABLE 4

'Starch digestion' in duodenal fistula dogs

SAMPLE	VOLUME COLLECTED CORRECTED FOR DILUTION	ACID			DILUTION	MILLIGRAM PER CENT MALTOSE		GRAMS MALTOSE RECOVERED	PER CENT DIGESTION	
		pH	Free Cl. U.	Total Cl. U.		At recovery	After incubation to indicate presence of active enzyme		At recovery	After incubation to determine amount of enzyme present
Dog no. 5. 400 cc. 7½ % farina cereal. No malt extract added. Recovered 184 cc. (46 %). Emptying time 80 minutes. Average of three determinations										
Control		7.0			1.00	204	280		4.5	6.2
30 minutes	64	4.3	3.5	11.0	0.83	277	490	0.18	6.2	10.9
60 minutes	109	3.2	7.0	18.0	0.69	480	512	0.52	10.6	11.4
Duodenal collection	173							0.70		
Into intestine	195							0.93		
Left in stomach	32							0.07		
Total	400							1.70	9.4	
Dog no. 5. 400 cc. 7½ % farina cereal containing 0.7 gm. malt extract. Recovered 258 cc. (64.5 %). Emptying time 55 minutes. Average of three determinations										
Control[1]		7.0			1.00	592	2030		13.2	45.0
10 minutes	95	4.8	7.5	13.5	0.79	932	1665	0.88	20.7	37.0
20 minutes	67	4.2	6.5	12.5	0.67	1670	2840	1.12	37.0	63.2
40 minutes	56	3.0	10.0	20.5	0.62	1733	2063	0.97	38.6	46.0
55 minutes	40	2.4	29.0	33.5	0.64	1780	1767	0.71	39.5	39.2
Duodenal collection	258							3.68		
Into intestine	136							1.94		
Left in stomach	6							0.02		
Total	400							5.64	31.3	

[1] Not introduced into stomach.

Comparison of human saliva with malt diastase. Two experiments were performed in which an amount of human saliva diastatically equivalent to the amount of malt diastase employed was added to the farina. One cubic centimeter of the human saliva used was equivalent to 140 mg. of the malt

TABLE 5

Effect of human saliva on starch digestion in duodenal fistula dog

Dog no. 5. 400 cc. 7½ % farina cereal containing 5 cc. human saliva. Recovered 214 cc.; emptying time 45 minutes; average of two determinations

SAMPLE	CORRECTED VOLUME	ACID			DILUTION	MILLIGRAM PER CENT MALTOSE			GRAMS MALTOSE RECOVERED	PER CENT DIGESTION		
		pH	Free Cl. U.	Total Cl. U.		At recovery	After incubation	Reactivate to pH 7.0 and incubated at 37°		At recovery	After incubating	Attempting to reactivate by readjusting to pH 7.0 and incubation at 37°
Control[3]		6.8			1.00	548	1784			12.2	39.7	[4]
10 minutes	75	5.8	....	10.0	1.00	784	1840	1910	0.59	17.4	40.9	42.4
25 minutes	52	5.9	....	7.5	0.92	1270	2260	2260	0.66	28.2	50.3	50.3
35 minutes	74	4.6	4.6	15.0	0.78	1320[2]	1420	1400	0.98	29.3[1]	31.5	31.1
45 minutes	13	2.9	35.0	55.0	0.63	1230	1240		0.16	27.3	27.6	
Duodenal collection	214								2.39			
Into intestine	171								1.91			
Left in stomach	15								0.18			
Total	400								4.48	24.9		

[1] Enzyme inactivated at this point.

[2] Enzyme inhibited at this pH.

[3] Not introduced into stomach.

[4] Figures in this column show that adjusting the pH of the duodenal contents to neutrality does not reactivate the inactivated ptyalin.

extract (comparisons made at pH 6.8). The results are shown in table 5, and they may be compared to the results in table 4, which were obtained using malt extract. It is to be noted that less maltose (approximately 20 %) was obtained with the diastatically equivalent amount of saliva. This was due to the fact that the ptyalin was inactivated at pH 4.5, and

malt extract acts longer in the stomach. Thirty-one and seven-tenths per cent of the active ptyalin passed into the duodenum during the first 25 minutes or before the acidity rose to the point of inactivation of ptyalin.

Is salivary or malt amylase inactivated by acid in the stomach reactivated in the duodenum? This was investigated because of Roger's ('07) claim that saliva inactivated by acid may be reactivated by traces of amylase. Bergeim ('26) found this not to be the case. Our results confirm Bergeim, who pointed out that Roger failed to control the electrolyte concentrations of his digestion mixtures.

Amount of malt amylase that passes into the intestine when ingested in milk and water with food in the stomach. On the complete duodenal fistula dogs cited above, two experiments were done in which it was found that when malt extract is ingested in water after a meal 75 % of the active enzyme passed into the duodenum within 10 minutes. Since, as is well known, milk does not pass into the duodenum so rapidly, it was decided to compare milk with water in two dogs with an incomplete duodenal fistula, an animal preparation which is more normal in regard to gastric evacuation than the former. Further, it was thought that the acid-buffering properties of the milk would retard the rise in pH of the gastric contents and prolong the action of the amylase in the stomach.

Three tests were performed on each of two dogs as follows: Five minutes after the ingestion of the cereal meal, 250 cc. of either milk or water containing 0.7 gm. of malt extract and the phenol red indicator was drunk by the dogs. The assay had to be modified to consider the reducing substances present in the milk. Continuous collections from the duodenal fistula were made, the various assays being made for enzyme, dilution, etc., at 15-minute intervals. With water the quantity of enzyme that passed into the duodenum during the first 30 minutes varied between 62 and 71 %. With milk during the same period from 34 to 54 % of enzyme was recovered from the duodenum. The acidity of the gastric contents rose

more slowly with milk than with water. As a result slightly more gastric digestion of starch occurred in the milk than in the water tests. The results of one of the tests are shown in table 6.

These results show again that if it is desired to have the enzyme pass through the stomach into the intestine rapidly, it is best to ingest the enzyme in water after eating a meal.

TABLE 6

Starch digestion and active enzyme recovered from intestine of duodenal fistula dog

400 cc. 7½ % farina ingested followed by drinking 250 cc. water or milk containing dye and 0.7 gm. malt extract

DOG NO. 15	VOLUME		pH CHANGE	POTENTIAL MALTOSE INTRODUCED IN FORM OF STARCH (GRAMS)	MALTOSE RECOVERED (GRAMS) FROM DUODENUM	PER CENT OF STARCH CONVERTED TO MALTOSE	PER CENT OF ACTIVE ENZYME RECOVERED FROM DUODENUM
	Collected from duodenum (cc.)	Corrected volume (for enzyme solution)					
Water							
At 30 minutes	235	157	6.5 –4.25	18.0	3.37	18.7	62.8
At 60 minutes	420	218	4.25–3.25		5.53	30.8	87.2
Milk							
At 30 minutes	215	135	6.5 –5.1	28.0[1]	5.04	18.0	54.0
At 60 minutes	330	207	5.2		8.97	32.0	83.0

[1] In farina 18.0 gm.; in milk — 10.0 gm.

Of course, if an excess of malt amylase is taken either in water or milk, a sufficient quantity will remain in the stomach to effect complete or almost complete gastric digestion of starch.

EXPERIMENTS ON HUMAN SUBJECTS

The experiments on the dogs were conducted primarily to obtain more crucial data upon which to base the interpretation of possibly less crucial data on human subjects. In the human subject such experiments are further complicated by the presence of salivary amylase, a factor that cannot be entirely controlled. Only graduate students in physiology accustomed to swallowing the gastric tube were employed so

that in some experiments an attempt to control the salivary factor might be made. The various assays made in the experiments on the dog, except those on duodenal chyme, were made on the gastric contents of the human subjects, samples being withdrawn in some instances and all of the contents in others.

Experiments in which only a limited quantity of saliva was swallowed. Tests were performed on eight subjects in which

TABLE 7

Gastric digestion of starch in man (rapid increase in acidity) subject J.G.

400 cc. 7½ % farina cereal. Without and with malt extract

Stomach evacuated and lavaged with acid prior to the digestion of a meal, the saliva formed after the ingestion of a meal being expectorated

SAMPLE	VOLUME COLLECTED (CC.)	CORRECTED VOLUME	ACID			DILUTION	MILLIGRAM PER CENT MALTOSE		PER CENT DIGESTION	
			pH	Free Cl. U.	Total Cl. U.		At recovery	After incubation	At recovery	After incubation
Control test—no malt extract										
In vitro[1]			6.5			1.00	584	856	13.0	19.0
15 minutes	30	26	2.7	15.0	25.0	0.87	856	984	19.0	21.8
30 minutes	30	19	1.9	45.0	52.5	0.65	1140	1310	25.4	29.2
45 minutes	30	14	1.8	45.0	53.7	0.48	1240	1220	27.6	27.1
With malt extract (0.7 gm.)										
In vitro control[1]			6.7			1.00	440	2200	9.8	48.8
10 minutes	30	32	3.8	5.0	12.5	0.75	1865	3270	41.6	72.6
20 minutes	30	18	2.2	17.5	30.0	0.61	2000	2170	44.4	48.3
30 minutes	30	16	1.8	25.0	35.0	0.55	2000	2125	44.4	47.3
45 minutes	30	15	1.4	55.5	72.5	0.51	1995	2105	44.3	46.8

[1] Not introduced into stomach.

the cereal meal (400 cc. 7.5 % farina) was subjected only to that amount of saliva swallowed during the ingestion of the meal, which required from 3 to 5 minutes, the malt extract being mixed with the meal just immediately before its ingestion. Prior to the ingestion of the meal the stomach was evacuated of its contents and then lavaged with 0.2 % HCl to inactivate any ptyalin present. After the ingestion of the meal the subject expectorated the saliva secreted. This was done to simulate a deficiency of salivary ptyalin.

Samples of the gastric contents were withdrawn at 10- or 15-minute intervals and assayed for reducing sugar, pH, enzyme, etc.; the phenol red indicated the dilution factor. One test was made with and one without adding malt extract. The response of three of the eight subjects is shown in tables 7, 8 and 9. The response of the eight subjects are summarized

TABLE 8

Gastric digestion of starch in man (moderate increase in acidity) subject J.B.

400 cc. 7½ % farina cereal. Without and with malt extract

SAMPLE	VOLUME COLLECTED	ACID			DILUTION	MILLIGRAMS MALTOSE PER CENT		PER CENT DIGESTION	
		pH	Free acid	Total acid		At recovery	After incubation	At recovery	After incubation
Without malt extract									
In vitro control[1]		7.0			1.00	272	240	6.1	5.3
10 minutes	40	5.9		8.0	1.20	960	1905	21.3	42.3
20 minutes	40	2.4	37.5	50.0	0.83	1240	1290	27.5	28.7
30 minutes	40	2.0	47.5	65.0	0.92	1190	1230	26.4	27.3
45 minutes	40	1.9	60.0	80.0	0.80	1170	1290	26.0	28.7
With malt extract (0.7 gm.)									
In vitro control[1]		6.5			1.00	652	3400	14.4	75.5
10 minutes	40	6.3		7.5	0.68	2720	4540	60.3	100.0
20 minutes	40	3.2	20.0	37.5	0.65	2860	2960	63.5	65.8
30 minutes	40	2.6	45.0	62.5	0.42	3430	3430	76.0	76.0
45 minutes	40	2.4	42.5	57.2	0.49	3100	3100	69.0	69.0
50 minutes	40	2.3	52.5	67.5	0.46	3130	3130	69.6	69.6

[1] Not introduced into stomach.

in table 10. In giving the per cent of digestion only the amount of gastric digestion of starch found is considered, because the amount of undigested starch that passed into the intestine was not and could not be accurately ascertained in normal subjects.

It was found that the amount of gastric starch digestion depended on the rate at which the acidity of the gastric contents rose, more starch digestion being obtained when malt extract was added in seven out of the eight subjects. The responses may be divided into three or perhaps four groups. A) In subjects 1, 2 and 3 the acidity rose rapidly to the point of inactivation of ptyalin, but not malt amylase. In subject 4,

TABLE 9

Gastric digestion of starch in man (delayed increase in acidity). Subject R.S.

400 cc. 7½ % farina cereal. Without and with malt extract

SAMPLE	VOLUME COLLECTED (CORRECTED FOR DILUTION)	VOLUME INTO DUODENUM	ACID			DILUTION	MILLIGRAMS MALTOSE PER CENT		PER CENT DIGESTION	
			pH	Free acid	Total acid		At recovery	After incubation to detect enzyme present	At recovery to detect amount of enzyme present	After incubation
Without malt extract										
In vitro control[2]			6.5	...	...	1.00	178	178	3.9	3.9
15 minutes	242	158	5.4	...	5.0	0.90	3290	4030	73.0	89.5
30 minutes	42	316	5.3	...	5.0	0.87	3800	4020	84.5	89.3
45 minutes[1]										
With malt extract (0.7 gm.)										
In vitro control[2]			6.5	...	...	1.00	712	1950	15.8	43.4
10 minutes	284	116	4.9	...	5.0	1.00	3090	5290	68.5	100.0
20 minutes	170	206	5.5	...	5.0	0.83	4300	5110	95.5	100.0
30 minutes	60	291	5.6	...	5.0	0.71	5100	5360	100.0	100.0
45 minutes[1]										

[1] Stomach empty.

[2] Not introduced into stomach.

the same type of response occurred, but the acidity rose somewhat more slowly yielding 36 % digestion with ptyalin and 78 % with malt amylase. B) In subject 5 the acidity at 45 minutes was as high as in those in the first group, but it rose initially more slowly, which accounts for the marked digestion with both the salivary and malt amylase. C) In subject 6 the acidity rose very slowly and the stomach emptied

rapidly which accounts for high values for gastric starch digestion. D) In subjects 7 and 8 the acidity rose to the lethal point of ptyalin and malt amylase at about the same time, so that malt amylase did not increase the gastric digestion of starch.

The early part of the curve of the rate of digestion in the salivary and malt amylase tests are quite identical. The malt amylase simply continues to act from 5 to 15 minutes longer in the stomach than salivary amylase.

TABLE 10

The effect of malt amylase on the gastric digestion of starch in man

Values represent the conditions present 45 minutes after ingestion of 400 cc. 7½ % farina cereal, with and without the addition of 0.7 gm. malt extract

SUBJECT	CONTROL			WITH MALT EXTRACT		
	pH	Per cent digestion at recovery	Active ptyalin	pH	Per cent digestion at recovery	Active amylase
1. (J.G.)	1.8	27.6	Absent	1.4	44.3	Absent
2. (J.B.)	1.9	26.0	Absent	2.4	69.0	Absent
3. (W.B.)	1.6	26.4	Absent	1.3	68.2	Absent
4. (J.V.)	4.1	36.7	Absent	4.0	78.5	Present
5. (R.B.)	2.0	73.4	Absent	2.1	98.0	Absent
6. (R.S.)[1]	5.3	84.5	Trace	5.6	100.0	Present dig. compl.
7. (D.M.)	3.0	54.8	Absent	1.5	58.2	Absent
8. (R.R.)	2.2	29.7	Absent	1.9	26.1	Absent
Approximate average		45			68	

[1] Stomach empty at 45 minutes, values given for 30 minutes.

In order to obtain more accurate data pertaining to the amount of active enzyme that passed into the duodenum, five of the subjects were given the meal under the above conditions and 10 or 15 minutes later the stomach was completely evacuated. A sample was kept for assay and the remainder injected into the stomach to be removed again 10 or 15 minutes later. The data are summarized in table 11 and show that a minimum of 42 % of the enzyme had passed into the duodenum before inactivation. In the hypochlorhydric subject 'R. S.' approximately all entered the duodenum in active

form. The average for the five subjects was 54 % which is less than in the experiments on dogs, because the acidity on the average rose more rapidly in our human subjects.

Human subjects: Experiments in which saliva was swallowed at all times. These experiments were performed to simulate normal conditions of eating. In the first experiment

TABLE 11

Summary of results on human subjects

SUBJECT	pH CHANGE DURING TEST PERIOD	VOLUME AT END OF TEST PERIOD		PER CENT DIGESTION OF STARCH IN STOMACH AT END OF TEST PERIOD	AVERAGE PER CENT DIGESTION OF STARCH ENTERING DUODENUM DURING TEST PERIOD	MALTOSE (GRAMS)			TOTAL PER CENT STARCH CONVERTED TO MALTOSE	PER CENT ACTIVE ENZYME ENTERING DUODENUM
		In stomach	Passed into duodenum			Recovered	Entered	Total		
J.B. (15) .... [1]	6.5–3.2	282	118	60	37	7.62	1.97	9.59	53.2	29.5
D.M. (15) .... [1]	6.5–3.2	230	170	30	20	3.11	1.53	4.64	25.8	42.5
R.S. (10)	6.5–5.6	284	116	69	42	8.83	2.19	11.02	76.9	90.0
(20)		141	259	96	83	6.10	7.52	13.62		
(30)		43	359	100	98	1.90	11.94	13.84		
R.R. (15) .... [1]	6.0–2.6	150	250	23	18	1.55	2.02	3.57	19.8	62.5
W.B. (15) .... [1]	6.0–2.7	215	185	45	29	2.81	2.19	5.00	27.8	46.2
								Average		54.3

Results based on the gastric starch digestion that occurred during the time that the pH was falling to a level near that at which malt amylase is inactivated (pH 2.4). In these experiments the stomach was entirely evacuated every 10 or 15 minutes, a sample taken for assay and the balance returned depending on the pH found.

[1] Data for other 15-minute periods not given because point of inactivation of enzyme has been reached prior to 20 minutes.

of this type the cereal meal with and without malt extract was ingested and all the saliva was swallowed, the malt extract being mixed with the cereal just before it was ingested. Only one experiment of this kind was done because the results were the same as in the above experiments on this subject, i.e., more starch digestion occurred when malt extract was

added. This was due to the fact that the acidity of the gastric contents destroyed the ptyalin of the swallowed saliva.

The following experiment was then performed on ten human subjects, eight of whom had been used in the above experiments. The test meal used consisted of 200 gm. of mashed

TABLE 12

Starch digestion in the human stomach when a mixed meal is ingested with and without the addition of malt amylase to milk consumed at intervals during the meal. Stomach completely evacuated at end of 2 hours

SUBJECT	pH AT END OF 2 HOURS	CLINICAL UNITS OF HCl		PER CENT DIGESTION OF STARCH	PRESENCE OR ABSENCE OF ACTIVE ENZYME IN STOMACH
		Free	Total		
Without malt amylase	4.10	32.5	62.5	92.3	Absent
J.B. With malt amylase	4.18	21.5	51.2	100.0	Present
Without malt amylase	4.10	32.5	67.5	85.7	Absent
W.B. With malt amylase	4.43	20.0	47.5	100.0	Present
Without malt amylase	3.70	40.0	67.5	100.0	Absent
R.R. With malt amylase	3.80	42.0	47.5	100.0	Present
Without malt amylase	4.26	22.5	62.5	100.0	Absent
L.L. With malt amylase	4.30	27.5	60.0	100.0	Present
Without malt amylase	2.82	65.0	115.0	100.0	Absent
R.B. With malt amylase	2.90	62.5	112.0	100.0	Absent
Without malt amylase	4.4	5.0	20.0	72.0	Present
M.P. With malt amylase	4.8	12.0	30.0	100.0	Present
Without malt amylase	3.0	70.0	112.5	94.2	Present
M.D. With malt amylase	2.9	68.0	115.0	95.0	Present
Without malt amylase	3.1	67.5	100.0	98.5	Absent
J.G. With malt amylase	3.3	62.5	96.0	100.0	Present
Without malt amylase	2.2	82.5	112.5	86.2	Absent
C With malt amylase	2.1	50.0	100.0	100.0	Absent
Without malt amylase	1.9	85.0	125.0	100.0	Absent
S With malt amylase	2.1	85.0	115.0	100.0	Absent

potatoes, 100 gm. of liver sausage, 30 gm. of butter and 250 cc. of milk. The milk was ingested during the meal. The malt extract (5.5 gm.), when used, was dissolved in the milk. The meal was ingested 12 hours after the last meal, all saliva being swallowed. Two hours later the entire gastric contents

were aspirated, the volume noted, and a sample used for pH and acidity determinations. One portion was immediately inactivated and the concentration of reducing sugar was determined immediately; and another sample was incubated for 2 hours with an excess of malt amylase to determine the per cent of starch digestion; still another portion was assayed for active malt amylase. From these data the percentage of gastric starch digestion was calculated, the total yield of reducing sugar from the meal having been previously determined.

The results are shown in table 12. It is to be noted that in four of the ten subjects (J.B., W.B., M.D. and C) the addition of malt amylase increased the gastric digestion of starch. In these subjects the pH of the gastric contents was sufficient to destroy ptyalin but not malt amylase. In the other subjects complete digestion of the starch occurred in the stomach without the addition of malt amylase. (Of course, some undigested starch entered the intestine.)

The interpretation of the foregoing results, we believe, is quite obvious. In the four of the subjects the ptyalin digestion of starch in the stomach was incomplete. An adequate amount of ptyalin was not present during the initial period of gastric digestion to effect complete gastric digestion of starch before the acidity rose to the point at which ptyalin is inactivated. In the other subjects the ptyalin was adequate to effect complete gastric digestion of starch before the acidity rose to the point of inactivation of ptyalin, and the addition of malt amylase was superfluous.

DISCUSSION

The observations on dogs are not considered to require discussion because the results are so clear cut and their interpretation so obvious. To our knowledge no previous work of this nature has been done on dogs. These observations on dogs form the basis of studies now under way on the effectiveness of orally administered malt amylase in the presence of pancreatic deficiency.

The observations on the human subjects may be discussed in the light of those of Bergeim ('26) and Müller ('01). Müller used rice mush as a test meal and found that from 59 to 80 % of the starch was rendered soluble. Using bread in four subjects, the figures observed by Müller varied from 50 to 77 %, the starch digestion being less when the bread was poorly masticated. Bergeim, using potatoes (six subjects) obtained figures varying from 54 to 84 % (average 76), and using bread (six subjects) obtained figures varying from 46 to 68 % (average 59). Bergeim requested his subjects not to swallow saliva prior to the meal and to masticate in a reasonably thorough manner. About half an hour after the first of the food was ingested and at 15-minute periods thereafter, samples were removed for assay. He added ferric hydrate to his meal for the same reason that we added phenol red. For cereal (farina) in our 'restricted salivary amylase control' experiments (table 10), the per cent of digestion (reducing sugars) varied from 26 to 84%, average 45. This is less than that observed by the above observers, because in these experiments we lavaged the stomach of our subjects and had them expectorate saliva after the meal to simulate a salivary deficiency. In the case of our mixed meal (table 12) in which potatoes (we used 200 gm.; Bergeim 100 gm.) served as the starch substrate, we observed from 72 to 100 % digestion of starch. These figures are higher than those of Bergeim, because protein (liver sausage and milk) was present in our meal which buffered acid and permitted the salivary amylase to act longer.

SUMMARY

1. When cooked starch containing malt extract was placed in the stomach of dogs with a pouch of the entire stomach and retained there and histamine injected to stimulate the secretion of the pouch, 20 % (corrected) of the starch was digested in 1 hour before the acidity rose to a point at which malt amylase is inactivated. Ninety-five per cent of the digestion occurred in the first 30 minutes.

2. A. In dogs with a complete duodenal fistula with the bile and pancreatic juice excluded, the addition of malt extract to a meal of cereal ('farina') appreciably facilitated gastric emptying because of the liquefying action of amylase. The addition of malt amylase increased the digestion of the starch from a control value of from 5 to 12 % to a value of from 30 to 65 % (average increase 370 %). About 87 % of the amylase passed into the duodenum in active form in these experiments. Variations in the per cent of starch digestion were directly correlated with the rate of evacuation and the rise in acidity of the gastric contents.

B. When a known quantity of malt amylase in water was drunk after the ingestion of a cereal meal, 75 % of the enzyme passed into the duodenum within 10 minutes the remainder becoming mixed with the gastric contents to promote the gastric digestion of starch (16 to 20 % increase). Thus, if it is desired to introduce malt amylase rapidly into the intestine in active form, this is best accomplished by taking the enzyme in water after a meal (vide infra).

3. A. The results obtained on dogs with an incomplete duodenal fistula and with pancreatic juice and bile excluded compared favorably with those on the complete duodenal fistula dogs, except that the percentage gastric digestion of starch was somewhat less on the average, varying from 30 to 40 %. This was due to the more rapid rise in gastric acidity in incomplete duodenal fistula dogs.

B. When a diastatically equivalent amount of human saliva was substituted for malt amylase, considerably more starch digestion resulted with malt amylase. This is due to the fact that ptyalin, which is inactivated at pH 4.5 does not act as long in the stomach as malt amylase which is inactivated at pH 2.5.

C. When the experiment cited under 2 B above was repeated on these dogs, which more closely simulate the normal, both milk and water being used as a solvent of the malt extract, it was found that with water from 69 to 71 % of the enzyme is obtained from the duodenum in 30 minutes, whereas with milk

34 to 54 % of the enzyme is obtained, because the milk does not leave the stomach as rapidly as water. The milk buffers acid and the acidity rises somewhat more slowly. However, with the administration of an excess of malt amylase little difference, if any, in the amount of gastric digestion of starch would obtain.

4. A. In experiments on human subjects performed so as to simulate a salivary deficiency, the addition of malt extract to a cereal meal definitely augmented the gastric digestion of starch in seven out of eight subjects. The variations observed depended upon the rate at which the acidity of the gastric contents rose.

B. In experiments designed to ascertain the amount of active malt amylase given with a cereal meal that passes into the intestine before the acidity rises to the lethal point of the enzyme, the minimum figure of 42 and a maximum of approximately 100 % was obtained, the average being 51 %.

C. In experiments in which salivary amylase was permitted to play its normal role and a mixed meal (mashed potatoes, milk, liver sausage and butter), containing a moderate amount of acid buffering substances was ingested after which milk with and without malt extract added was drunk, it was found that the addition of malt amylase increased the gastric digestion of starch in four of ten subjects. In six the gastric digestion of starch was completed by ptyalin and the addition of malt amylase was superfluous. Thus, in the larger percentage of normal human subjects who partake of a mixed meal containing a moderate amount of substances which buffer acid, the gastric starch digestion resulting from salivary amylase is practically complete.

5. Neither salivary nor malt amylase after being inactivated in the stomach are reactivated in the intestine. This confirms Bergeim.

COMMENT

In a minority of normal persons the ingestion of malt amylase adds to the salivary amylase swallowed with a meal and exerts an additional influence on the gastric digestion of starch. This influence is greater in the presence of a deficiency of salivary amylase. However, in the presence of an adequate secretion of pancreatic (amylopsin) and intestinal amylase (the amylase of succus entericus), the addition of malt amylase to the meal may be considered as superfluous. However, in the presence of a deficiency of pancreatic amylase and of an inadequate secretion of saliva, which might be due to defective ptyalin formation or to the bolting of food in a person whose gastric acidity rises rather quickly to the lethal point of ptyalin, the addition of malt amylase to the meal is definitely indicated by our results. Actual tests on dogs and on patients suffering a deficiency of pancreatic secretion are necessary to substantiate this reasonable a priori deduction.

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A TECHNIC FOR DETERMINING THE RATE OF ABSORPTION OF FATS[1, 2]

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ONE FIGURE

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INTRODUCTION

The rate of absorption of fat may be measured directly by determining the amount of fat remaining in the alimentary tract at intervals after feeding fat, or indirectly by following the appearance of fat in the blood stream. The latter measurement is, of course, influenced by factors other than the actual absorption of fat from the tract as, for example, the rate of metabolism, deposition of fat in the tissues, and mobilization of fat from the tissues. Obviously, the direct method is preferable if one is interested primarily in absorption. In this laboratory we have adapted the technic as developed by Cori ('25) for sugars to the determination of the rate of absorption of fats. In applying this technic certain modifications had to be made which are described in detail in this report.

Two methods for determining blood fat were also tried, the chylomicron method of Gage and Fish ('24) and the haemolipokrit method of Rückert ('31). Each of these has its own intrinsic value, but neither was satisfactory for our purpose, as will be pointed out later.

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[2] Published with the permission of the director of the Wisconsin Agricultural Experiment Station, Madison.

EXPERIMENTAL

Direct method of determining fat absorption

Fasting. Adult male rats 4 to 7 months of age and weighing between 200 and 400 gm. were fasted 48 hours before they were fed a fatty meal. It was found necessary to fast the animals this length of time to empty the stomach and small intestines of food previously eaten. No amount of fasting up to 1 week emptied the caecum. During the 48-hour fast distilled water only was available, and the animals lost 9.4 % of their weight.

Feeding. In order to feed a measured quantity of fat the animals were lightly anesthetized with ether, a mouthpiece was placed behind the incisors, and a small (no. 8) urethral catheter inserted into the stomach. By means of a 2 cc. hypodermic syringe attached to the catheter 1.5 cc. of melted fat was delivered directly into the stomach. In a similar manner 1.5 cc. of fat was delivered from the syringe directly into a beaker and weighed. The average value of several such weighings was used in all calculations as the weight of the fat fed. These weights checked to within 0.01 gm.

Removing the contents of the alimentary tract. When the desired time had elapsed after feeding the fat the contents of the intestinal tract were removed. The rats were decapitated, the abdominal cavity opened, and ligatures placed at the oesophageal sphincter and at the caecal-colonic juncture. The stomach, small intestine, and caecum were removed immediately from the body cavity. For convenience in handling, ligatures were also tied at the pyloric sphincter, at the ileo-caecal valve, and at the middle of the intestine. The stomach and caecum were severed from the intestine, and the intestine was cut into two parts. By means of a hypodermic syringe each section was filled with distilled water. After 10 minutes the contents of the stomach and intestine were emptied into one beaker and the contents of the caecum into another. The sections were then filled with petroleum ether to dissolve the remaining fat, and after another 10 minutes

the contents were emptied into the appropriate beakers. The stomach and caecum were cut wide open and washed thoroughly with a jet of water and petroleum ether. The intestine was stripped manually to remove the last vestige of food. The contents of the stomach and intestine and the contents of the caecum were extracted separately because the caecum often contained a viscous material that made extraction in large volume difficult. Petroleum ether was used as the fat solvent because of its insolubility in water, its good solvent properties, and its relative physiological inertness.

Extraction of fat from the alimentary residues. The extraction of fat from the collected washings was continued with petroleum ether. The washings were transferred to a 125 cc. separatory funnel, petroleum ether added to make the total volume approximately 25 cc., and extracted vigorously. In all, four extractions were made. The ether extracts were combined (total volume about 100 cc.) and 1 gm. of anhydrous sodium sulfate added. The extract was then filtered into a tared beaker and the sulfate washed thoroughly. After standing over night the beakers were placed in a vacuum oven at 92 to 95°C. for 3 hours. For the first ½ hour the pressure was held at 610 mm. Hg; it was then lowered to 85 mm. Hg and maintained at 85 mm. Hg during the remaining 2½ hours. After cooling in a desiccator, the beakers were weighed.

To test the accuracy of this technic, groups of rats were fed 1.5 cc. of melted fat, killed immediately, and an attempt made to recover the fat by the method described. In all cases the percentage recovery was between 95 and 100 %. For instance, ten such animals when fed a hydrogenated fat showed a mean percentage recovery of 97.5 % (range 96.3 to 100.0 %). Another group of ten rats fed corn oil showed a mean percentage recovery of 97.8 % (range 95.0 to 99.1 %). It was apparent that the method used was sufficiently accurate for biological work of the character undertaken.

A small but significant amount of fat was always found in the caecum. In most cases the mean weight of the caecal fat

increased up to 6 or 8 hours after a meal of fat and then decreased. For example, after feeding 1.3550 gm. of butter fat, the mean amounts of fat extracted from the caecal contents at 2, 4, 6, 8 and 12 hours after feeding were 0.0319, 0.0363, 0.0483, 0.0426 and 0.0312 gm., respectively. The mean amount of fat found in the caecal contents after 48 hours of fasting was 0.0086 gm.

TABLE 1

Individual experiments showing the percentage of fat absorbed by adult male rats fed halibut liver oil

RAT NO.	GRAMS OF HALIBUT LIVER OIL FED	ABSORPTION TIME	WEIGHT OF FAT IN CONTENTS OF STOMACH AND INTESTINES	WEIGHT OF FAT IN CAECUM CONTENTS	CORRECTED WEIGHT OF FAT RECOVERED [1]	TOTAL WEIGHT OF FAT ABSORBED	PERCENTAGE OF FAT ABSORBED
		hours	*gm.*	*gm.*	*gm.*	*gm.*	
♂ 324	1.3656	2	0.8980	0.0250	0.9008	0.4648	34.0
♂ 329	1.3656	2	0.8548	0.0210	0.8536	0.5120	37.5
♂ 330	1.3656	2	0.9639	0.0166	0.9583	0.4073	29.8
♂ 349	1.3656	2	0.8228	0.0098	0.8104	0.5552	40.7
♂ 499	1.3656	2	0.8616	0.0220	0.8614	0.5042	36.9
♂ 500	1.3656	2	0.7824	0.0116	0.7718	0.5938	43.4
♂ 501	1.3656	2	0.9754	0.0140	0.9672	0.3984	29.1
♂ 507	1.3656	2	0.8107	0.0187	0.8072	0.5584	40.8
♂ 323	1.3656	2	0.6558	0.0092	0.6428	0.7228	52.9
♂ 508	1.3656	2	0.7193	0.0147	0.7118	0.6538	47.8
♂ 509	1.3656	2	0.8280	0.0186	0.8244	0.5412	39.6
Mean							39.4±1.6

[1] The values in this column are the sum of the weights given in the two preceding columns minus the correction factor, 0.0222 gm.

Correction factor. In calculating absorption values it was essential to know how much fat was present in the intestinal tract after the 48-hour fast. A group of seven rats was, therefore, fasted, killed, and the fat extracted by the method described above. The mean weight of the fat obtained by this procedure was 0.0222 gm. This correction was applied in each case before the percentage absorption was calculated.

Results. In table 1 are given the complete data for a group of eleven rats fed 1.5 cc. of halibut liver oil and killed 2 hours later. At first glance it might appear that the range, 29 to 53,

is large, but throughout all of our experiments on absorption similar variations were found. A range greater than this was almost never encountered, and usually the extreme values fell within the range of M $\pm$ 10 to 12. The probable errors as shown in table 2 were typical of all experiments. Apparently these variations are an expression of the innate differences in the ability of the animals to absorb fat.

The effect of the anesthetic. In order to determine whether or not etherization had any effect upon the rate of fat absorption, groups of rats were kept under ether for 30 minutes, 10 minutes, 5 minutes, and 1 minute before feeding, and the percentage of fat absorbed in 4 hours determined. A fourth group of rats was not anesthetized but was offered 1.5 cc.

TABLE 2

The effect of ether upon the percentage of fat absorbed in 4 hours by adult male rats fed 1.5 cc. of corn oil

TREATMENT	NUMBER OF RATS	MEAN PERCENTAGE ABSORPTION
No ether	8	61±0.9
Etherized 1 minute	10	58±0.9
Etherized 5 minutes	6	57±1.9
Etherized 10 minutes	6	52±1.1
Etherized 30 minutes	6	37±2.7

of fat in a small dish. If the animal did not consume the fat in 10 minutes, as determined by weighing the dish before and after feeding, it was not included. Forced feeding without etherization was tried, but we came to the conclusion that light anesthesia was much less disturbing to the animal than the excitement caused by forced feeding.

It is apparent from the results (table 2) that prolonged etherization had an effect upon absorption. However, the shorter periods of anesthetization, 1 and 5 minutes, had no significant effect. (Statistical analyses have shown that for a group of ten animals a mean difference of around 10 % is necessary to give significance.)

Our attempts to secure voluntary consumption of small amounts of fat in a short enough period of time to avoid the

introduction of a serious error were found highly impractical. It was decided, therefore, to anesthetize the rats the shortest possible time and to use a standard procedure for all the animals. The method adopted was as follows:

Three cubic centimeters of anesthesia ether were poured onto one-half of a paper towel which had been placed in the bottom of an open ether can of 4-liter capacity. The can was covered immediately with a heavy glass cover and the ether allowed to vaporize for 3 minutes at room temperature. The animal was then introduced and removed just as soon as it had relaxed. The fat was fed at once. Invariably the animal recovered consciousness and walked around the cage with perfect equilibrium 1 to 2 minutes after the fat was administered.

The amount of fat fed. Gross trial experiments showed that as much as 1.5 cc. of fat could be fed successfully to our adult male rats and still leave sufficient residue of fat in the intestine after 6 hours for satisfactory analysis.

In view of the conflicting statements (Cori, Cori and Goltz, '29; Cori, Villiaume and Cori, '30; Cori, '30; MacKay and Bergman, '33; Magee and Reid, '31; Pierce, Osgood and Polansky, '29; and Wilson and Lewis, '29) concerning the effect of the amount of sugar fed upon its rate of absorption, the amount of fat fed to our rats was increased and the results observed. Previously 1.5 cc. (1.3434 gm.) of a commercially hydrogenated vegetable oil had been fed to groups of ten rats each and the percentage of fat absorbed at 2, 4, 6, 8 and 10 hours determined. We now fed twice as much, namely, 2.6868 gm. of the same fat and determined the absorption. The results are shown in table 3. Greater irregularities were encountered when 3 cc. instead of 1.5 cc. of fat were fed. As a matter of fact, a number of the animals on the larger amount had diarrhea and had to be discarded. Apparently 3 cc. of fat, when administered according to our technic, was more than our rats could digest. Consequently 1.5 cc. of fat was fed in all subsequent experiments.

The effect of age. To determine if age is a factor influencing the rate of absorption of fat, nine rats that were 10 months of age were fed in comparison with others. The mean percentage of fat absorbed by these animals was 3 % lower than the general average of our rats 4 to 7 months old—a difference which is not significant. Again, a group of five young rats 40 to 50 days of age were tested. In 4 hours these rats absorbed 56 ± 2.4 % of the fat fed, whereas animals that were 4 to 7 months of age absorbed 54 ± 1.6 % of the same fat. Obviously, age, within the limits of our experiment, namely 4 to 7 months, did not influence the rate of absorption to any great extent.

TABLE 3

The percentages of fat absorbed by groups of adult male rats fed 1.5 cc. or 3 cc. of fat

TIME	FED 1.5 CC. OF FAT		FED 3 CC. OF FAT	
	Mean number of milligrams absorbed	Mean percentage absorbed	Mean number of milligrams absorbed	Mean percentage absorbed
hours				
2	357.6±20.6	26.6±1.5	509.7±77.8	19.1±0.9
4	723.2±21.2	53.8±1.6	780.5±34.1	29.0±1.2
6	918.8±24.2	68.5±1.7	1183.5±39.1	44.0±1.3
8	1155.9±16.6	86.0±1.3	1383.4±45.9	51.4±1.7
10	.....	.....	2143.9±38.7	79.7±0.5[1]
12	1325.3± 3.5	98.6±0.3	2463.1±14.7	91.6±0.6

[1] This group contained only seven animals.

Sex as a factor in absorption. To determine the influence of sex a comparison of the absorption of adult male and female rats was made. The females lost on the average 9.9 % and the males 9.4 % of their weight during the 48-hour fast. The mean differences between the percentages of fat absorbed by male and female rats (table 4) are not statistically significant.

The effect of pregnancy. To test the effect of pregnancy on the absorption of fat five females between the second and third week of pregnancy were fed 1.5 cc. of a hydrogenated fat and killed after 4 hours. The results were no different

from those obtained from a group of eleven male rats similarly treated; the females absorbed a mean of 50.3 ± 3.7 % of the fat fed; the males, 52.8 ± 2.4 %.

Seasonal variations. For the most part our experiments were done during the spring and summer of 1933. In the fall of 1934 after a particularly hot summer a large number of female rats in the stock colony were not breeding normally.

TABLE 4

The percentage of fat absorbed by male and female rats at 2, 4, 6, 8 and 12 hours after feeding 1.5 cc. of fat

	NUMBER OF RATS	MEAN WEIGHT	MEAN PERCENTAGE FAT ABSORBED
		gm.	
Absorption time 2 hours			
Male	25	286	26.3±0.57
Female	15	225	23.9±0.65
Absorption time 6 hours			
Male	27	273	64.2±0.94
Female	19	215	63.3±1.25
Absorption time 8 hours			
Male	7	283	85.7±1.63
Female	2	216	83.0 ...
Absorption time 12 hours			
Male	10	267	99.4±0.15
Female	6	218	99.2±0.30

Rather than discard them outright, an attempt was made to use them in fat-absorption tests. At the time, when controls were run on the base fat, a hydrogenated vegetable oil, a mean of 45.8 ± 1.6 % of the fat was absorbed by a group of ten female rats, whereas the mean obtained previously for this fat was 53.8 ± 1.6 %. The experiment was repeated immediately using six male rats. The mean percentage absorption was 52.3 ± 0.9 %, a figure comparable to the 1933 value. The absorption of corn oil was checked also. The

1933 value for male rats fed corn oil was 58.3 ± 0.9 %; the 1934 value, 61.0 ± 1.5 %. Apparently the physical condition of the female rats that we were attempting to use was affecting their ability to absorb fat. We, accordingly, found it necessary to confine our comparisons to experiments carried out within periods of limited time and on animals in a similar physiological condition.

Correlation coefficients between body weight, body surface, length of intestines, and the grams of fat absorbed. Cori ('25) assumed that the amount of sugar absorbed by a rat is proportional to body weight. He, therefore, expressed his results as a coefficient calculated by the formula $\frac{A \times 100}{W \times T}$, wherein A represents the milligrams absorbed, W the weight of the animal after fasting, and T the time of absorption in hours. This coefficient of absorption represents the milligrams absorbed per 100 gm. of body weight per hour of time. Pierce, Osgood and Polansky ('29) and MacKay and Bergman ('33) state that the rate of absorption of glucose appears to be more closely related to body surface than to body weight. They substitute body surface for body weight in the formula above and express the coefficient of absorption as the milligrams of glucose absorbed per square centimeter of body surface per hour. Cori used young and growing animals, whereas we used adults. In order to determine to what extent the amount of fat absorbed by our animals was dependent upon body weight, body surface, or intestinal surface, correlation coefficients were calculated between these variables. The formula of Carman and Mitchell ('26), $S = 11.36\ \text{wt.}^{2/3}$, was used in calculating body surface. The intestinal surface was assumed to be proportional to the length of the intestine. It was found that the measured length of the removed intestine varied greatly with the method of removing it from the body. However, if the mesentery was pulled off as the organ was removed and the intestines stretched carefully, more drastic treatments, even stripping manually to remove the contents, did not alter the length more than a few centimeters.

The correlation coefficients and their probable errors at the different absorption periods are shown in table 5. The correlations between the grams of fat absorbed and the length of the intestines show that there is no striking relation between these factors. The mean length of the intestine for twenty-one female rats upon which this measurement was taken was 93.9 cm. with a range of 65 to 108 cm. A similar measurement on fifty male rats was 93.3 cm. with a range of 69 to 115 cm. From the physiological point of view it is quite possible that the conditions of our experiment in no way taxed the ability of the intestinal surface to absorb fat into the blood stream. Moreover, the intestinal surface is only one of the possible limiting factors in the absorption

TABLE 5

Correlation coefficients between body weight, length of intestine, body surface, and the grams of fat absorbed

ABSORPTION TIME IN HOURS	NUMBER OF OBSERVATIONS	CORRELATION BETWEEN BODY WEIGHT AND GRAMS OF FAT ABSORBED	CORRELATION BETWEEN LENGTH OF INTESTINE AND GRAMS OF FAT ABSORBED	CORRELATION BETWEEN BODY SURFACE AND GRAMS OF FAT ABSORBED
2	15	-0.32 ± 0.16	-0.36 ± 0.15	-0.32 ± 0.16
4	41	$+0.27\pm0.10$	$+0.17\pm0.07$	$+0.27\pm0.10$
6	41	$+0.38\pm0.09$	$+0.32\pm0.09$	$+0.27\pm0.10$
8	38	$+0.36\pm0.09$	$+0.25\pm0.10$	$+0.41\pm0.09$

of fat; it may under proper circumstances play a role of secondary importance to the secretion of digestive juices, the rapidity and ease of emulsification, the concentration of fat in the blood stream, and many others.

Of the correlation coefficients given in the table only one of them is three times as large as its standard deviation, namely the correlation between body surface and the grams of fat absorbed at 8 hours. This correlation is not supported by similar correlations at the other absorption periods, so no especial significance can be attributed to it. Since no very definite or striking correlations exist between the measured variables, there is no logic in relating absorption to any one of them. Our results, therefore, are expressed simply as percentage of the fat absorbed.

The determination of blood fat

Chylomicron method. Various attempts were made to increase the accuracy of the chylomicron method, but no measurable success was attained. The technic finally adopted was essentially that proposed by Gage and Fish ('24) in their original article. Other micro methods of determining blood fat are reviewed by Man and Gildea ('32).

The rats were fasted 24 hours and then fed 0.5 cc. of fat by stomach tube. Five-tenths cubic centimeter was fed instead of 1.5 cc. to keep the number of chylomicrons appearing in the blood from becoming too numerous to count. A small drop of blood from the end of a rat's tail was touched to a coverslip (no. I, 1 sq.cm.) which was immediately placed in contact with a glass slide and weighted with a 500-gm. weight for 10 seconds. The size of the drop taken was such that the blood film covered about three-fourths of the area of the coverslip. Duplicate samples were mounted on one slide and the edges sealed with mineral oil. Counting was usually completed within an hour after the collection of the sample. A Leitz microscope equipped with a dark field, a 1/12 oil immersion objective, a 10 × ocular, and an ocular net 5 mm. × 5 mm., graduated in 0.5 mm., were used. A field was considered suitable for counting when about one-fourth of the area of the net was not occupied with blood cells and was without fibrin clots and/or oxyhemoglobin crystals. The number of moving particles in each of four ¼ sq.mm. was counted in a given field. Ten fields were counted and an average value calculated for the number of chylomicrons in 1 sq.mm.

Obviously, there are many errors in this technic. When the number of chylomicrons is large, the accuracy of counting them with their brownian movement is greatly diminished. Consequently, we attempted to dilute the blood. By means of suitable pipettes dilutions of 1:200, 1:400, 1:800 and 1:1600 of blood in 1 % acetic acid solution were made. A 1 % acetic acid solution was used in preference to a physiological saline because it laked the red blood cells and thus

helped to clear the field. When counts were made, the expected relation between the different dilutions was by no means always obtained.

Both clotted blood and oxalated blood were centrifuged and counts made on the serum. The interference of the blood cells was thereby eliminated, but when the cells themselves were suspended in saline solution and viewed under the microscope, the mount was teeming with chylomicrons.

Attention was then directed toward the use of a counting chamber in order to insure a definite depth of solution. The Petroff-Hauser counting chamber was found to be as satisfactory as any, but by focusing up and down at different levels we were able to bring almost any number of chylomicrons into view.

To test the effect of fasting the animal upon the number of chylomicrons in the blood, three fasting periods were used—16, 24 and 48 hours. No differences in the response of the animals could be detected.

In another experiment rats were fasted 24 hours, anesthetized, and kept under the influence of ether for 15 minutes before they were fed 0.5 cc. of fat. No effects due to the prolonged etherization could be demonstrated.

Rats were exercised either 15 or 30 minutes before feeding fat by allowing them to swim in a tank of water at 35°C. Other animals were massaged gently for similar periods before feeding. Although the number of animals was too few to draw final conclusions, it appeared that both exercise and massage caused a slight increase in the number of chylomicrons.

The effect of temperature was studied by counting the chylomicrons in the blood of rats kept at 14°C. and at 48°C. during the fasting period. After feeding 0.5 cc. of fat, counts were made in the usual manner. It appeared that the low temperature did not affect the chylomicron response, but the higher temperature seemed to reduce it. No differences were noted at the end of the fasting period before fat was fed.

It was thought that if a fat-free diet could be substituted for the fasting period, the response of the rats would more nearly approach a normal physiological response. The number of chylomicrons in the blood was as low when a fat-free diet had been fed for 48 to 72 hours as when the animal was fasted. When fat was fed to the animals on a fat-free diet, the peak of the curve was lower and tended to occur later than when the animals were fasted.

The number of chylomicrons appearing in the blood of rats fed a fat-free diet for 72 hours and then fed 0.5 cc. of various fats was determined. Typical curves for two of the fats tested, namely butter oil and halibut liver oil, are given in figure 1. There was a definite tendency for the curves to be of the same general outline when a given rat was fed the same fat at different times. Variations in successive responses of one rat were not as great as variations between the responses of different rats receiving the same fat. However, variations were so great that we were unable to demonstrate any differences in the rate of absorption. We concluded, therefore, that the chylomicron method makes an interesting demonstration of an increase in blood fat following the ingestion of fat, but that for our purpose it had little or no quantitative value.

Haemolipokrit method. The haemolipokrit method of Rückert ('31) was found to be much more satisfactory as a quantitative measure than the chylomicron method. The technic used was essentially that described in detail by Herrmann, Ames and Tapke ('34).

The animals were fasted 48 hours, fed 1.5 cc. of fat, and at the proper intervals thereafter anesthetized with ether and 3 cc. of blood drawn by heart puncture. The blood was allowed to stand 15 minutes at 37°C. to facilitate clotting before it was centrifuged. The serum obtained was either clear or slightly milky due to the presence of fat particles.

Duplicate analyses on the same sample of serum gave concordant results, but the variations from rat to rat were fairly large. For instance, one group of ten rats bled 4 hours after

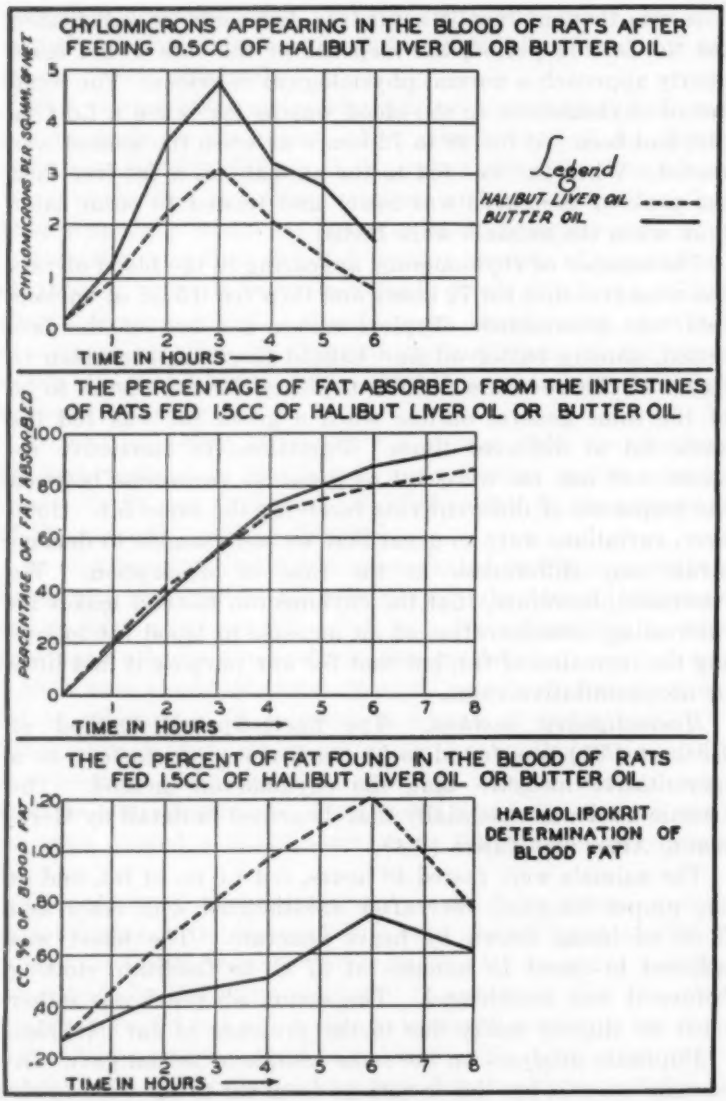

Fig. 1 Each point on the curves is the mean of ten or more individual values.

receiving halibut liver oil gave values for blood fat ranging from 0.62 cc. % to 1.22 cc. % with a mean value of 0.97 ± 0.04 cc. %. Whereas these variations are greater than those obtained when the rate of absorption of fat from the intestine was measured directly, they are perhaps to be expected, since one is measuring absorption from the intestine as influenced by the factors of metabolism.

In figure 1 are given the blood fat curves for groups of rats fed halibut liver oil or butter oil.[3] It will be noted that the chylomicron and haemolipokrit curves for blood fat do not substantiate each other. The haemolipokrit values only can be considered significant and indicate a definite difference in the rate of appearance of fat in the blood when butter oil and halibut liver oil are fed. Determinations of the rate of absorption of these two fats show that they disappear from the alimentary tract at approximately the same rate. According to Hughes and Wimmer ('35) the shorter chain, more soluble fatty acids are absorbed directly into the portal circulation and are not resynthesized into fat as they pass through the intestinal wall. If this is the case, it might account for the smaller amounts of butter oil found in the blood. It is also possible that the shorter chain fatty acids are metabolized or stored more rapidly in the body than the longer chain acids.

Since we were interested in determining the rate of absorption of fat from the tract rather than in studying blood fat and since the results on blood fat did not parallel the data on absorption, we limited our work to the direct method of analyzing the intestinal residues.

[3] Preparation of butter oil. Butter was melted and the butter fat separated by decantation; 240 gm. of the butter fat was dissolved in 1500 cc. of absolute alcohol at 45°C. and then cooled to 1°C. The mixture was filtered and the alcohol removed from the filtrate by distillation in a partial vacuum with nitrogen at 40 to 45°C.; 33 gm. of butter oil were obtained.

SUMMARY

A method for determining the comparative rate of absorption of fat from the alimentary tract has been described in detail. The method involves the feeding of a definite quantity of fat and subsequent analysis of the intestinal residues. Concomitant use of the chylomicron method and the haemolipokrit method for determining blood fat did not give comparable results. The former was found to have little or no quantitative value. The haemolipokrit method gave concordant results on duplicate samples of serum but was not found satisfactory for studying the rate of absorption of fats because different fats appeared in the blood at rates quite different from those with which they disappeared from the alimentary tract.

No definite or striking correlations between the grams of fat absorbed and the variables—body weight, body surface, or length of intestines—were found.

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THE COMPARATIVE RATE OF ABSORPTION OF DIFFERENT FATS [1, 2]

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INTRODUCTION

The literature dealing with the absorption of fats has been adequately reviewed by Verzar ('33). Of the various investigators, Holmes and co-workers (Deuel and Holmes, '22; Holmes, '18 a, '18 b, '19 a, '19 b; Holmes and Deuel, '20, '21; and Langworthy and Holmes, '15, '17 a, '17 b) have specialized in studies of absorbability of fats. So far, however, little work has been done on the comparative rate of absorption of different fats. It is this phase of the physiology of fats with which our present experimental work deals. We found that certain fats are more rapidly absorbed than others, but whether or not rapidity of absorption is physiologically advantageous is beyond the scope of this paper. We merely wish to present the data at hand.

EXPERIMENTAL

The technic used in determining the rate of absorption of fats has already been described by Irwin, Steenbock and Templin ('36). Briefly, it consisted of a modified Cori ('25) method in which adult male rats fasted for 48 hours were lightly anesthetized with ether and fed 1.5 cc. of melted fat

[1] The authors wish to express their sincere appreciation to Lever Brothers Company whose financial support made this work possible.
[2] Published with the permission of the director of the Wisconsin Agricultural Experiment Station, Madison.

by stomach tube. At intervals thereafter the animals were killed and the contents of the alimentary tract analyzed for fat.

The fats studied were lard, corn oil, two hydrogenated vegetable oil shortenings designated as A and B, respectively, butter fat, butter oil, halibut liver oil, and cod liver oil. These were considered as representative of a wide variety of fats. They were fed to groups of ten or more rats and the percentage of fat absorbed at 2, 4, 6, 8 and 12 hours determined. All the fats except the butter fat and the butter oil were commercial products.[3] The butter fat used was prepared from butter as churned from sweet cream. No salt or coloring matter was added. The butter was melted and the butter fat separated from the water and casein by decantation. The butter oil was prepared by dissolving butter fat in absolute alcohol at 45°C. and then cooling the mixture to 1°C. The mixture was filtered and the alcohol removed from the filtrate by distillation in a partial vacuum with nitrogen at 40 to 45°C. The oil as obtained from 240 gm. of butter fat dissolved in 1500 cc. of alcohol weighed 33 gm. All of the solid fats were melted and fed at temperatures a few degrees above their melting points.

The mean percentages of fat absorbed together with their probable errors for the different absorption periods are given in table 1. The results of 342 individual experiments are represented by these data. It will be seen that the percentage of fat absorbed at the different absorption periods was practically the same when lard, corn oil, or the hydrogenated shortenings were fed. Butter oil, halibut liver oil, and cod liver oil were absorbed at similar rates but were definitely more rapidly absorbed than the group just mentioned. The rate of absorption of butter fat falls somewhere between these two groups.

[3] The fats and oils used were those available commercially. Hydrogenated shortening A was manufactured by hydrogenating cottonseed oil to an iodine number of approximately 70. The saponification number was 196.0. For shortening B corresponding data were not available.

Corn oil was chosen as the fat with which to compare butter fat, butter oil, halibut liver oil, and cod liver oil in calculating the statistical significance of the differences in the percentages of fat absorbed because the percentage of corn oil absorbed at the different absorption periods was

TABLE 1

The mean percentages of fat absorbed by rats fed 1.5 cc. of fat[1]

KIND OF FAT FED	ABSORPTION TIME				
	2 hours	4 hours	6 hours	8 hours	12 hours
Lard	24.1±0.8	57.0±1.5	67.5±1.5	92.3±0.9	97.8±0.4
Corn oil	28.9±0.8	58.3±0.9	71.4±2.1	94.4±0.7	97.9±0.3
Shortening B	27.1±1.8	52.8±2.4	71.1±1.5	85.6±1.2	99.6±0.1
Shortening A	26.6±1.5	53.8±1.6	68.5±1.7	86.0±1.3	98.6±0.3
Butter fat	36.2±1.6	60.3±1.2	77.2±2.0	91.2±1.1	97.4±0.4
Butter oil	37.4±2.3	71.0±1.2	86.4±1.7	95.6±1.0	.....
Halibut liver oil	39.4±1.6	70.2±2.0	78.1±1.3	85.4±0.9	.....
Cod liver oil	40.8±1.4	67.7±1.9	79.7±1.5	89.2±0.7	98.2±0.4

[1] All of the animals were adult male rats. Each figure is the mean of ten or more individual experiments except in the case of the 12-hour group fed cod liver oil, which figure is a mean of only five experiments.

TABLE 2

Mean differences, standard deviations of the mean differences, and $\frac{MD}{\sigma MD}$ *for butter fat, butter oil, halibut liver oil, and cod liver oil compared with the base fat, corn oil*

	2 HOURS			4 HOURS			6 HOURS		
	MD	σMD	$\frac{MD}{\sigma MD}$	MD	σMD	$\frac{MD}{\sigma MD}$	MD	σMD	$\frac{MD}{\sigma MD}$
	%			%			%		
Butter fat and corn oil	7.2	2.7	2.7	2.0	1.9	1.0	5.8	4.3	1.3
Butter oil and corn oil	8.5	3.6	2.4	12.7	2.2	5.7	15.0	4.0	3.7
Halibut liver oil and corn oil	10.5	2.6	4.0	11.9	3.3	3.6	6.7	3.6	1.8
Cod liver oil and corn oil	11.9	2.4	4.9	9.4	3.1	3.0	8.3	3.8	2.2

as high as lard or the hydrogenated fats. If the differences between butter oil, halibut liver oil, or cod liver oil and corn oil are significant, similar differences between these three fats and lard or the hydrogenated fats are also significant. The mean differences together with their standard deviations and the quotients $\frac{MD}{\sigma MD}$ at 2, 4 and 6 hours are given in table 2.

At 8 and 12 hours so much of the fat had been absorbed that the differences were no longer accurately representative of the rate of absorption. Therefore, these differences were not included in the table. Parenthetically, it may be stated that for biological work of this nature a mean difference that is two to three times as large as its standard deviation is usually considered statistically significant. It is evident that there is a real difference in the rates of absorption of butter oil as compared with corn oil (table 2). There is likewise a difference between the rates of absorption of halibut liver oil or cod liver oil and corn oil. The differences in favor of butter fat are suggestive but are not statistically significant.

Examination of table 1 will reveal that butter oil, halibut liver oil, and cod liver oil were absorbed at approximately the same rate for the first 4 hours, but at 6 and 8 hours halibut liver oil and cod liver oil lagged behind. Since it has been shown that certain hydrocarbons and higher alcohols, especially cholesterol, are not completely absorbed (Channon and Collinson, '28; Channon and Devine, '34; and Mahdi and Channon, '33), it was thought that these differences might be due to the presence of a larger residue of unsaponifiable matter from cod liver oil and halibut liver oil. Saponification of butter oil and halibut liver oil showed that butter oil contained 1.5 % of unsaponifiable matter, whereas halibut liver oil contained 13 %. To test out this point, 1.5 cc. of these two fats were fed to groups of six male rats each. After a 6-hour absorption period the animals were killed and the percentage of unsaponifiable matter in the pooled residues from the intestines of each group determined. The residue from the animals fed butter oil contained 5.3 % unsaponifiable matter, whereas those fed halibut liver oil contained 17.4 %. Apparently the unsaponifiable fraction of the oils was more slowly absorbed than the glycerides.

To test this point further unsaponifiable matter prepared from halibut liver oil was added to butter oil to make it contain 12.4 % of the same. The mixture was then fed to groups

of six animals each which were killed at 4, 6 and 8 hours, respectively. The percentage of butter oil absorbed under these conditions was 60 % at 4 hours, 69 % at 6 hours, and 78 % at 8 hours. This makes it seem probable that the larger amount of unsaponifiable matter present in halibut liver oil and in cod liver oil accounted for at least part of the lag in the absorption of these oils in comparison with butter oil.

In order to follow more closely the changes that take place in a fat as digestion proceeds, the intestinal residues from each group of rats fed cod liver oil were analyzed for unsaponifiable matter, acid number, and iodine number for various absorption periods (table 3). The data presented

TABLE 3

The percentage of unsaponifiable matter and the acid value expressed as percentage of oleic acid in the residues extracted from the digestive tract 2, 4, 6 and 8 hours after feeding cod liver oil

	ORIGINAL COD LIVER OIL	INTESTINAL RESIDUES AFTER FEEDING COD LIVER OIL			
		2 hours	4 hours	6 hours	8 hours
Percentage unsaponifiable matter	2.6	3.2	7.9	6.0	8.4
Acid value as percentage oleic acid	0.7	11.9	17.4	15.6	16.1
Iodine number—Hanus	154.0	...	150.0	...	...

confirm those obtained with halibut liver oil, namely that the unsaponifiable matter was absorbed less readily than the fat itself. It is apparent also that the hydrolysis of fat into fatty acids and glycerol proceeded more rapidly than the absorption of these compounds until such time as a balance between the rate of hydrolysis and absorption was established. The change in iodine value was insignificant.

At the 2-hour absorption period butter fat was more rapidly absorbed than corn oil, but at the 4- and 6-hour periods similar differences were not apparent (table 1). Since butter fat contains a larger percentage of short chain fatty acids than corn oil and since these acids are somewhat water-soluble, it is possible that they were absorbed more rapidly than the long chain fatty acids, and, therefore, a

difference occurred at 2 hours which did not persist throughout the absorption period. To test this possibility corn oil and butter fat were fed again to groups of thirteen rats each and the percentage of fat absorbed after 2 hours determined a second time. The residues from the gastro-intestinal tract were pooled and saponification and Reichert-Meissl numbers determined. The data obtained previously for the percentage absorption of these two fats (table 1) were confirmed by this experiment, butter fat being 36 % absorbed and corn oil 32 % absorbed. Analysis of the residual butter fat from the tract gave a saponification number of 224 and a Reichert-Meissl number of 20.13, whereas the original butter fat gave values of 230 and 27.17, respectively. Although these differences are not large, they indicate that the higher percentage of short chain fatty acids is a possible explanation of the more rapid rate of absorption of butter fat, which occurs at the 2-hour absorption period only.

When 1.5 cc. of fat was fed to the rats, the 4-hour absorption period appeared to be the one most suitable for comparing the rates of absorption. At this time digestion is well under way, the after effects of administration of the fat—if any—have undoubtedly been dissipated, and the diminished concentration of the fat is probably not yet a serious factor. Accordingly, we used the 4-hour absorption period for many of the fats studied. The results are shown in table 4. Whether or not another sample of any one of these fats would have shown a similar absorption rate cannot be stated, as we used only one sample. With individual variations of the magnitude encountered a mean difference of approximately 10 % is necessary for statistical significance. It should be borne in mind, therefore, that many of these fats (table 4) do not differ greatly in their absorption rates. There is, however, a very decided difference in the rate of absorption of oleo stock (36 %) and olive oil (63 %).

It is well known that fatty livers can be produced by feeding diets containing a relatively high percentage of fat. We became interested in determining whether or not there is any

difference in the production of fatty livers by different fats and whether such differences, if they occurred, could be associated with absorption rates. Seven groups of six rats each (male rats weighing 45 to 65 gm.) were fed a diet composed of crude casein 18, brewers' yeast 8, salts (Wesson, '32) 5, cooked starch 39, and fat 30. Vitamins A and D were supplied by 1 drop of halibut liver oil fed to each rat each week. The fats used were lard, butter fat, oleo stock, shortening A, coconut oil, olive oil, and a mixture of cod liver oil and lard, one to two.

TABLE 4

Mean percentage of fat absorbed together with the probable errors, at a 4-hour absorption period

KIND OF FAT FED	NUMBER OF RATS	PERCENTAGE OF FAT ABSORBED
Raw linseed oil	9	67.0±0.9
Olive oil	10	63.4±1.8
Whale oil	9	62.1±1.3
Soya bean oil	10	58.5±1.4
Peanut oil	9	58.3±1.7
Rancid lard	10	53.8±1.6
Cottonseed salad oil	10	53.7±1.6
Coco butter	9	47.9±1.1
Coconut oil	9	47.4±1.6
Palm oil	10	37.4±1.5
Oleo stock	10	35.8±1.0

The animals were given these diets for a period of 3 weeks. They were then killed and the livers analyzed for fat. The mean percentages of fat expressed as linoleic acid found in the livers of the several groups were as follows: lard 15.0 ± 0.7, butter fat 14.7 ± 0.4, oleo stock 16.1 ± 0.3, shortening A 14.5 ± 0.6, coconut oil 19.2 ± 0.5, olive oil 16.8 ± 0.5, and the mixture of cod liver oil and lard 12.4 ± 0.8.

None of these differences is very marked. Possibly the lower fat content of the livers of rats fed cod liver oil can be attributed to the larger amount of nitrogenous constituents in this oil. However, within the scope of this experiment no very striking differences occurred.

SUMMARY

The percentage of fat absorbed from the alimentary canal of rats was determined at 2, 4, 6, 8 and 12 hours after feeding definite quantities of fat. It was found that: 1) partially hydrogenated vegetable oils, as sold commercially for home and bakers' use, were absorbed as rapidly as lard or corn oil, and 2) that butter oil, halibut liver oil, and cod liver oil were absorbed uniformly at a more rapid rate than lard, corn oil, or the partially hydrogenated fats.

Eleven other samples of fat tested after a 4-hour absorption period could be arranged in the following descending order of their percentage absorption: linseed oil, olive oil, whale oil, soya bean oil, peanut oil, rancid lard, cottonseed oil, cocobutter, coconut oil, palm oil, and oleo stock. Although the difference between any of these fats and the one immediately preceding or following in the list may not be significant, certainly there were very real differences in the absorption rates of those widely separated.

Besides differences in the character of the fats themselves, it appears that the quantity of unsaponifiable matter in a crude fat may influence the rate of absorption of the total.

No very striking differences occurred in the production of fatty livers when diets containing 30 % of lard, butter fat, oleo stock, partially hydrogenated vegetable fat, coconut oil, or olive oil were fed to young, growing rats.

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